The effects of concentrate composition and sequence of allocation on the metabolism and performance of growing sheep

Thesis

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THE EFFECTS OF CONCENTRATE COMPOSITION AND SEQUENCE OF ALLOCATION ON THE METABOLISM AND PERFORMANCE OF GROWING SHEEP

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I declare that this thesis has been composed entirely by myself and it has not been accepted in any previous application for a degree. The work, of which it is a record, has been done by myself. Quotations have been distinguished by quotation marks, and sources of information have been specifically acknowledged.

Jane Richardson.

ABSTRACT

Four experiments were carried out to investigate the effects of concentrate composition and sequence of allocation on the metabolism and performance of growing lambs. In the first experiment five feed ingredients were characterised using the *in situ* incubation technique. The degradability constants determined were then used in the construction of a spreadsheet which enabled prediction of hourly nitrogen and organic matter release from the diets to be used in subsequent experiments. In the second experiment 48 lambs were fed at a restricted level diets containing either barley or sugar beet pulp as the main energy source. Within each energy source the sequence of allocation of the individual ingredients was altered to give a pattern of nitrogen and organic matter release within the rumen which was synchronous, intermediate or asynchronous. Lambs fed the barley based diets deposited significantly more fat in the whole body than lambs on the sugar beet based diets ($p<0.001$) and lambs on the asynchronous diets tended to deposit less fat in the whole body than lambs on the synchronous or intermediate diets ($p<0.1$). There were significant differences between energy sources and degree of synchrony in the pattern of rumen and plasma nitrogen metabolites. In the third experiment the same diets were fed to 24 lambs and the effects on nutrient digestibility and microbial protein supply were assessed. Lambs on the sugar beet based diet showed significantly higher digestibilities of organic matter and neutral detergent fibre than lambs on the barley based diet, however the lambs on the barley based diet had a significantly higher level of purine derivative excretion ($p<0.05$) and a significantly higher calculated microbial protein supply ($p<0.05$). In the fourth experiment the barley based diets used in the previous experiments were fed *ad libitum* to 24 lambs. No significant effects on growth rate or carcass characteristics were seen and there were few significant differences in rumen or plasma metabolites. Overall, energy source within the diet had a greater effect on growth and metabolism of lambs than degree of synchronisation of nitrogen and organic matter supply to the rumen.

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LIST OF ABBREVIATIONS

AA	Amino acid
AFRC	Agriculture and Food Research Council
ARC	Agricultural Research Council
ATP	Adenosine triphosphate
BHB	β-hydroxy butyrate
DM	Dry matter
DUP	Digestible undegradable protein
EE	Ether extract
ERDP	Effective rumen degradable protein
FME	Fermentable metabolisable energy
GE	Gross energy
GH	Growth hormone
IGF-1	Insulin-like growth factor - 1
ME	Metabolisable energy
MP	Metbolisable protein
N	Nitrogen
NDF	Neutral detergent fibre
OM	Organic matter
OMADR	Organic matter apparently degradable in the rumen
RDP	Rumen degradable protein
TCA	Tricaboxcylic acid
VFA	Volatile fatty acid

CHAPTER 1. LITERATURE REVIEW.

1.1. INTRODUCTION.

Ruminant rationing systems in use today have been developed and refined over many years. Over the past 25 years several new systems for estimating the protein requirements of ruminant animals have been published. In the U.K. this has culminated in the publishing of the Metabolisable Protein (MP) system (AFRC 1992), which is based on the principles of ARC (1984) and introduces the concept of fermentable metabolisable energy (FME) in place of metabolisable energy (ME) and of effective rumen degradable protein (ERDP) and digestible undegradable protein (DUP) in place of rumen degradable protein (RDP) and undegradable protein (UDP). As microbial protein outflow from the rumen can account for 0.7 to 1.0 of the animals supply of absorbed amino acids (AFRC, 1992), and volatile fatty acids (VFA) can contribute 0.8 of absorbed energy (France and Siddons 1993), then factors influencing microbial growth are of major importance in all rationing systems. A common characteristic of rationing systems currently in use is that they consider nutrient requirements on a daily basis. However recent work has shown that considering the supply of nutrients to the rumen on an hourly basis may result in improved efficiency of microbial protein synthesis (Herrera-Saldana *et al.*, 1990a; Sinclair *et al.*, 1993,1995).

Sinclair *et al.* (1993) adopted Czerkwaski's (1986) value of 25gN/kg organic matter truly digested in the rumen as being the optimum ratio for microbial protein synthesis, and used it to calculate a 'synchrony index' (SI) which indicates the degree of synchrony of a particular feed based on hourly degradation of organic matter and protein within the rumen. Feeding sheep diets formulated to be synchronous resulted in increased efficiency of microbial protein synthesis (Sinclair *et al.*, 1993, 1995) whilst Witt *et al.* (1999a) reported that feeding synchronous diets to growing lambs resulted in improvements in live weight gain and feed conversion efficiency (FCE). However, other workers have found no significant

effect of synchrony on either microbial efficiency (Henning *et al.*, 1991, 1993; Kolver *et al.*, 1998) or animal production (Aldrich *et al.*, 1993; Kolver *et al.*, 1998; Shabi *et al.*, 1998).

Most studies conducted to evaluate the effects of dietary synchrony on microbial protein production or animal performance have compared diets formulated using different feed ingredients. Therefore the effects, or lack of, may have been due to some aspect of the diets that was not characterised rather than to synchrony. Few studies have been conducted to evaluate the effect of using alterations in the sequence of allocation of the same feedstuffs to alter the degree of synchrony of the diet, on microbial growth or animal performance.

1.2. CARBOHYDRATES IN RUMINANT DIETS

The majority of polysaccharides supplied in the ruminant diet fall into one of two major groups: structural polysaccharides which form the plant cell walls, and are considered as the "fibrous" fraction of the diet, and plant storage polysaccharides, such as starch and fructosan (Chesson and Forsberg, 1997).

1.2.1. Plant cell walls.

1.2.1.1. Chemical structure of plant cell walls

The polysaccharides which form plant cell walls are highly resistant to digestion, and it is the presence of rumen microorganisms which possess the ability to degrade these structural polysaccharides which allows the ruminant to utilise high fibre diets (Chesson and Forsberg, 1997). The polysaccharides contained in cell walls belong to one of three groups: cellulose, hemicellulose and pectin.

Cellulose.

Cellulose is the single major component of plant cell walls and is composed of long unbranched chains of glucose residues joined by β- 1,4 linkages (Chesson & Forsberg, 1997). Fibrils are formed by hydrogen bonding between adjacent cellulose microfibrils (Akin, 1984) and between cellulose microfibrils and arabinoxylans, xyloglucans and glucomannans which are cellulose-like in their conformation (Carpita and Gibeaut, 1993).

Hemicellulose.

Hemicellulose is defined as those polymers not extracted by hot water or chelating agents, but which are soluble in alkali (Chesson and Forsberg, 1988). In primary monocotelydon cell walls these are predominantly arabinoxylans, which are based on β-1,4 linked chains of

xylopyranosyl units with single glucuronic acid residues as side chains (Chesson & Forsberg, 1988).

Pectin

These polysaccharides are based on chains of α–1,4 linked galacturonic acid residues, and are found in far higher quantities in dicotelydon cell walls than monocotelydons (Chesson 1985; Flint and Forsberg, 1995). The bulk of pectic substances are found outside the cell wall, in the middle lamella region, and are therefore more susceptible to microbial digestion than cellulose or hemicellulose (Chesson, 1985).

1.2.1.2. Digestion of plant cell walls

The first stage in the ruminal digestion of plant cell walls is the attachment of the microbes to the fragments of plant material (Akin, 1984; Cheng *et al.*, 1983/84). It is mainly the cellulolytic bacteria which colonise the fragments, doing so in areas where the outer surface is damaged in some way (Akin, 1984; Wilson and Mertens, 1995). It has been suggested that the activities of the rumen fungi may reduce particle size or weaken the structure of the fibre, thus rendering it more open to bacterial attack (Akin *et al.*, 1989). The major cellulolytic bacteria include *Ruminococcus albus, R. flavefaciens, Butyrivibrio fibrisolvens* and *Fibrobacter succinogenes* (Van der Linden *et al.*, 1984; Cheng *et al.*, 1983/84; Chesson and Forsberg, 1997). The cellulase activity of rumen bacteria has been studied most thoroughly in *Fibrobacter succinogenes*, which is known to produce at least 11 different polysaccharidases (Chesson and Forsberg, 1997). The celluose degrading enzymes of non-rumen fungi and bacteria have been more intensively studied than those of rumen microorganisms (Béguin and Aubert, 1994), and it seems that in any cellulose degrading system a high degree of synergy is required between components of the enzyme system (Flint and Forsberg, 1995). The cellulolytic bacteria are highly sensitive to pH and growth is severely inhibited at pH6.0 or less (Mould and Ørskov, 1983a). Therefore the addition of highly fermentable concentrate to a forage diet will decrease the digestibility of the forage,

due to the rapid fermentation of the starch, and consequent reduction in pH (Mould *et al.*, 1983). Mould *et al.* (1983) also reported a 'carbohydrate effect' on cellulolysis in the rumen. When a rapidly fermentable energy source (barley) was added to forage based diets, the drop in cellulolysis could only be partly explained by the drop in pH. Mould *et al.* (1983) suggested that part of the decrease in cellulolysis is due to adaptation of the rumen microflora such that the readily fermentable carbohydrate can be degraded, which results in a decrease in the numbers of cellulolytic microorganisms.

Xylan forms a major component of hemicellulose, which itself makes up 37-48% of plant cell walls (Chesson *et al.*, 1986; Flint and Forsberg, 1995), and xylanases are widely distributed among the rumen bacteria (Flint and Forsberg, 1995). Many rumen bacteria produce more than one type of xylanase, which work cooperatively to fully degrade the oligomers released by the polysaccharide degrading enzymes (Flint and Forbserg, 1995). Hemicellulase and xylanase activities have also been found in rumen protozoa (Williams and Coleman, 1985). Pectolytic activity has been found in both the bacteria and the protozoa of the rumen (Chesson, 1980), however the rumen fungi seem to exhibit very little pectolytic activity (Williams and Orpin, 1987b). The pectolytic enzymes divide into two main groups; pectin esterases which catalyse the removal of ethanol, and depolymerising enzymes, which can be either hydrolases or lyases (Chesson and Forsberg, 1988). The bacteria active in the breakdown of structural carbohydrates produce acetic acid as the main fermentation end product, therefore diets high in fibrous materials give rise to rumen VFA mixtures high in acetic acid.

1.2.2. Plant Storage Polysaccharides.

1.2.2.1. Chemical structure of plant storage polysaccharides

Starch consists of two different polymers of glucose, amylose and amylopectin (Whistler and Daniel, 1984; Flint and Forsberg, 1995). Amylose consists of a linear chain of several hundred α-1,4 linked glucose units (Flint and Forsberg, 1995). Amylopectin also consists of

chains of α-1,4 linked units, but approximately one in 20-25 glucose units has an α- 1,6 linkage (Chesson and Forsberg, 1997). Amylose and amylopectin are stored in the plant in the form of starch granules. The size of the granules varies from less than 1μm, which are found in chloroplasts and are known as "leaf starch", to the much larger granules used for long term storage, which are found in stems, seeds, roots and tubers (French, 1984). The rate at which starch is digested in the rumen depends more on the structure of the surrounding tissue than on the starch itself (Chesson and Forsberg, 1997). The first barrier to starch digestion is the pericarp, or outer coat of the grain, and feed processing methods, or mastication by the animal is normally sufficient to disrupt this layer (Beauchemin *et al.*, 1994). The protein matrix surrounding the starch granule must also be disrupted to allow the starch to be digested (Chesson and Forsberg, 1997). In cereals this is readily achieved by the proteolytic bacteria, and is not an obstacle to starch breakdown (McAllister *et al.*, 1990a, b). However in other feedtsuffs such as maize and sorghum, the protein matrix is highly resistant to microbial attack (McAllister *et al.*, 1993).

1.2.2.2. Digestion of plant storage polysaccharides

Starch is utilised by many strains of rumen microorganisms, including the protozoa and fungi as well as bacteria. The amylolytic bacteria include *Prevotella ruminocola*, *Streptococcus bovis*, *Ruminobacter amylophilus* and *Succinomonas amylolytica* (Russell and Hespell, 1981; Marounek and Bartos, 1986; Chesson and Forsberg, 1997). Starch is broken down rapidly by amylases and other carbohydrases to maltose and ultimately to glucose monomers (Chesson and Forsberg, 1997). Fructosan also occurs in plants as a storage polysaccharide and consists of β–linked fructose units (Chesson and Forsberg, 1997). It is rapidly and completely degraded in the rumen by organisms including some species of protozoa (Williams, 1986; Ziolecki *et al.*, 1992). The protozoa engulf starch granules and take up soluble sugars which are stored within the protozoa and slowly metabolised thereby rendering them unavailable for bacterial fermentation (Schwartz and Gilchrist, 1975; Mackie *et al.*, 1978).

The carbohydrate monomers produced from the digestion of polysaccharides are metabolised into pyruvate (Baldwin, 1965; France and Siddons, 1993) and further to acetate, propionate and butyrate (Figure 1.1). The relative amount of each VFA produced is determined by the presence or otherwise of the different species of rumen bacteria, which is principally determined by the type of substrate available for fermentation (France and Siddons, 1993). Acetic acid always predominates, particularly on diets high in structural carbohydrate, and as the proportion of concentrate in the diet increases, the proportion of propionate rises, and that of acetic acid falls (France and Siddons, 1993). The VFA are absorbed directly through the rumen wall, their relative absorption rates being influenced by a number of factors, including the total concentration and ratios of the VFA, rumen pH and rate of blood flow through the rumen epithelium (van Houtert, 1993; Dijkstra *et al.*, 1993).

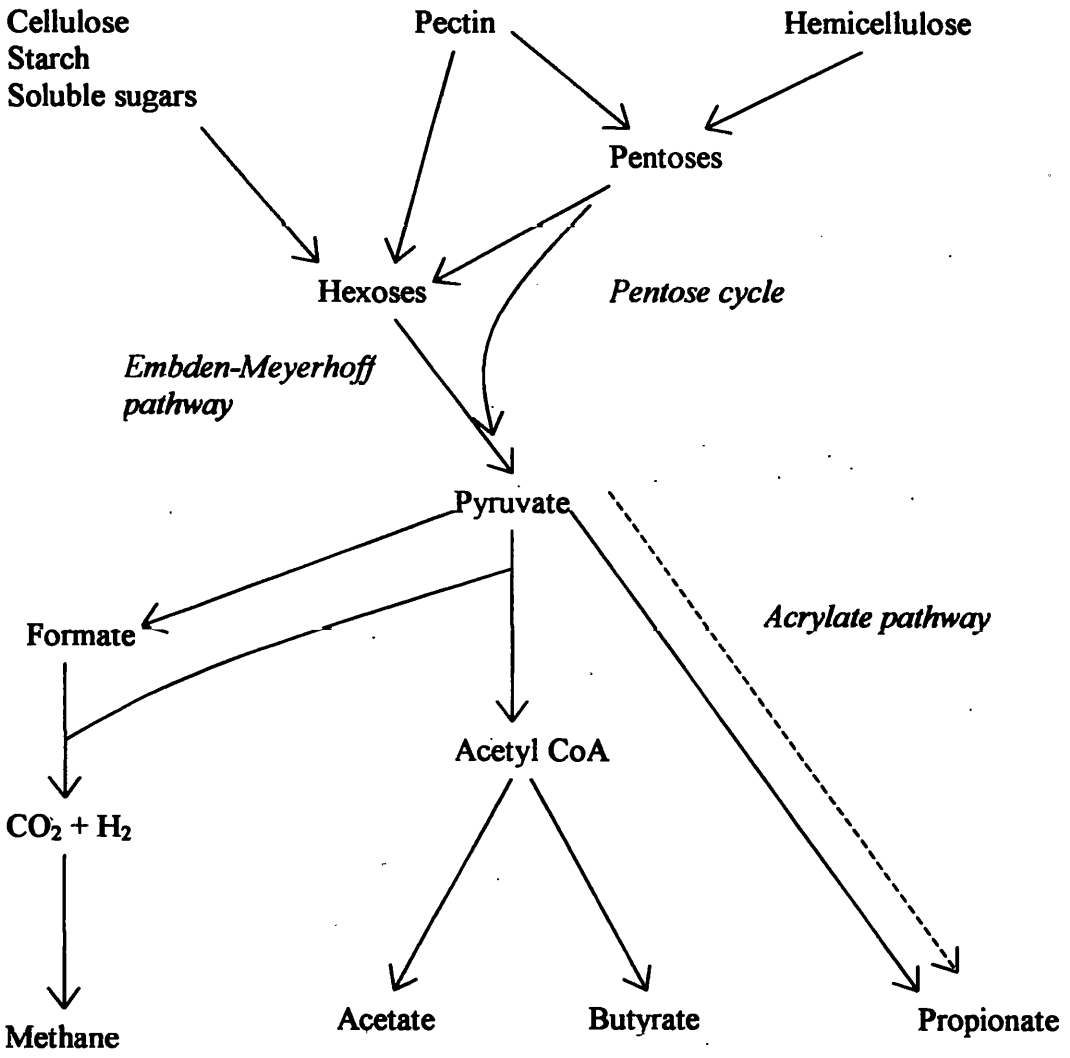


Figure 1.1. Schematic represntation of the major pathways of carbohydrate metabolism in the rumen (France and Siddons, 1993).

1.2.3. Effects of feed processing on carbohydrate degradability

The physical and chemical processes involved in diet manufacture have been reported to alter the degradability of carbohydrates. The findings of some of the work carried out to investigate the effects of various processes on digestibility are summarised in Table 1.1. The main factors in improvement of starch digestibility appear to be; 1) the disruption of the outer coat of the grain, by physical processing, and 2) disruption of the starch granules (Theurer, 1986). Physical processing of cereals disrupts the outer tissues of the grain, and allows entry of the rumen microorganisms, thereby increasing the susceptibility of the grain to degradation (Campling, 1991). Heat treatment causes swelling of the starch granules and gelatinisation of the starch, which renders it more digestible (Tait and Beames, 1988; Campling, 1991). Treatment with alkali (NaOH or ammonia) has a similar effect to rolling or crushing in that it allows entry of the rumen microorganisms. This is achieved through swelling of the outer starch particles leading to a disruption of the seed coat and partial gelatinisation of the outer starch granules (Barnes and Orskov, 1982).

Table 1.1. The effects of feed processing on digestibility of cereals.

Cereal	Process	Result	Reference
Corn	↓ particle size	↑ non-structural carbohydrate digestibility	Lykos and Varga (1995)
	Steam flaking	↑ non-structural carbohydrate digestibility	
	Formaldehyde	↓ dry matter degradability	Fluharty and Loerch (1989)
Barley	Expansion	↓ effective dry matter digestibility	Prestlokken (1999)
	Ammonia	↑ rumen organic matter digestibility	Mandell *et al.* (1988)
	Rolling	↑ rumen organic matter digestibility	
	NaOH vs. rolling	Higher OM digestibility on rolled than NaOH	Barnes and Orskov (1981)
High moisture barley	Rolling	↑ DM and starch digestibility	Rode *et al.* (1986)
	Ammonia	↑ DM and starch digestibility	
	Urea	↑ DM and starch digestibility	
Oats	Expansion	↓ effective dry matter digestibility	Prestlokken (1999)
	Rolled	↑ DM degradability	Moran (1986)
	NaOH	↑ DM degradability	
Wheat	Cracked	↑ DM digestibility ↓ NDF digestibility	Low and Kellaway (1983)
	Ammonia	↑ DM degradability ↑ NDF digestibility	
Straw	Ammonia	↑ DM amd OM digestibility	Orskov *et al.* (1983)

1.3. NITROGEN-CONTAINING COMPOUNDS IN RUMINANT DIETS.

Nitrogen enters the rumen from a number of sources, the most abundant of which is dietary protein (Wallace *et al.*, 1997). However in forage a considerable fraction of the N is present in non-protein forms, such as free amino acids, amides, nucleotides and peptides (Ried, 1994). Approximately half of forage protein is soluble, and has a relatively constant amino acid composition (Ried, 1994). Legumes contain globulins which are soluble in salt solution, and therefore easily degradable in the rumen, wheras cereals contain prolamines and glutelins, which are insoluble in salt solutions, and therefore relatively slowly degraded in the rumen (Lee, 1976; Krochko *et al.*, 1990). Nitrogen may also be supplied to the rumen as urea from nitrogen recycling.

1.3.1. Protein digestion.

Protein supplied in the diet is usually hydrolysed rapidly by the microorganisms in the rumen to peptides and amino acids which are further degraded to ammonia (Brock *et al.*, 1982; Cotta and Hespell, 1984). The rate and extent of protein breakdown depends on a number of factors. In general soluble proteins are broken down more rapidly than insoluble proteins, although this is dependant on the secondary and tertiary structure of the molecule (Henderickx, 1976; Mahadevan *et al.*, 1980). The greater the degree of cross linking, the greater the stability of the molecule, thus cleavage of disulphide bonds results in a more rapid breakdown of the protein (Wallace & Kopecny, 1983) and introduction of artificial cross linkages for example by heating or formaldehyde treatment, decreases the digestibility of the protein (Cozzi *et al.*, 1995; Lykos and Varga, 1995). Diets high in soluble carbohydrate tend to promote proteolytic activity in the rumen (Siddons & Paradine, 1981; Nugent *et al*, 1983) as it is the amylolytic rather than the cellulolytic bacteria which show proteolytic activity (Siddons and Paradine, 1981). The three species considered to be the

major proteolytic organisms are *Ruminobacter amylophilus*, *Butyrivibrio fibrisolvens* and *Prevotella ruminocola* (Cotta and Hespell, 1984; Morrison and Mackie, 1996).

The rumen protozoa are also known to have some proteolytic activity (Ushida *et al.*, 1991), although this is mainly confined to particulate proteins such as chloroplasts rather than soluble proteins (Mangan & West, 1977). They have also been shown to significantly affect the turnover of bacterial protein by engulfing entire bacterial cells, and so 'locking' them up in the rumen (Morrison and Mackie, 1996). The metabolism of bacterial protein seems to be the major proteolytic function of the protozoa (Wallace & McPherson, 1987). The fungi present in the rumen are also known to possess some proteolytic activity, but their exact role *in vivo* is not yet known (Wallace *et al.*, 1997).

The polypeptides released from ingested protein are broken down by the rumen microbes via a biphasic mechanism involving dipeptidyl peptidase and dipeptidases (Figure 1.2). When there is sufficient energy available to fuel biosynthesis, the released amino acids and peptides are incorporated directly into microbial protein (Wallace *et al.*, 1997). However, when energy is unavailable, or the rate of peptide breakdown exceeds the rate of uptake by the microbes, excessive ammonia is produced in the rumen (Wallace, 1994; Wallace *et al.*, 1997), leading to an inefficiency in nitrogen retention.

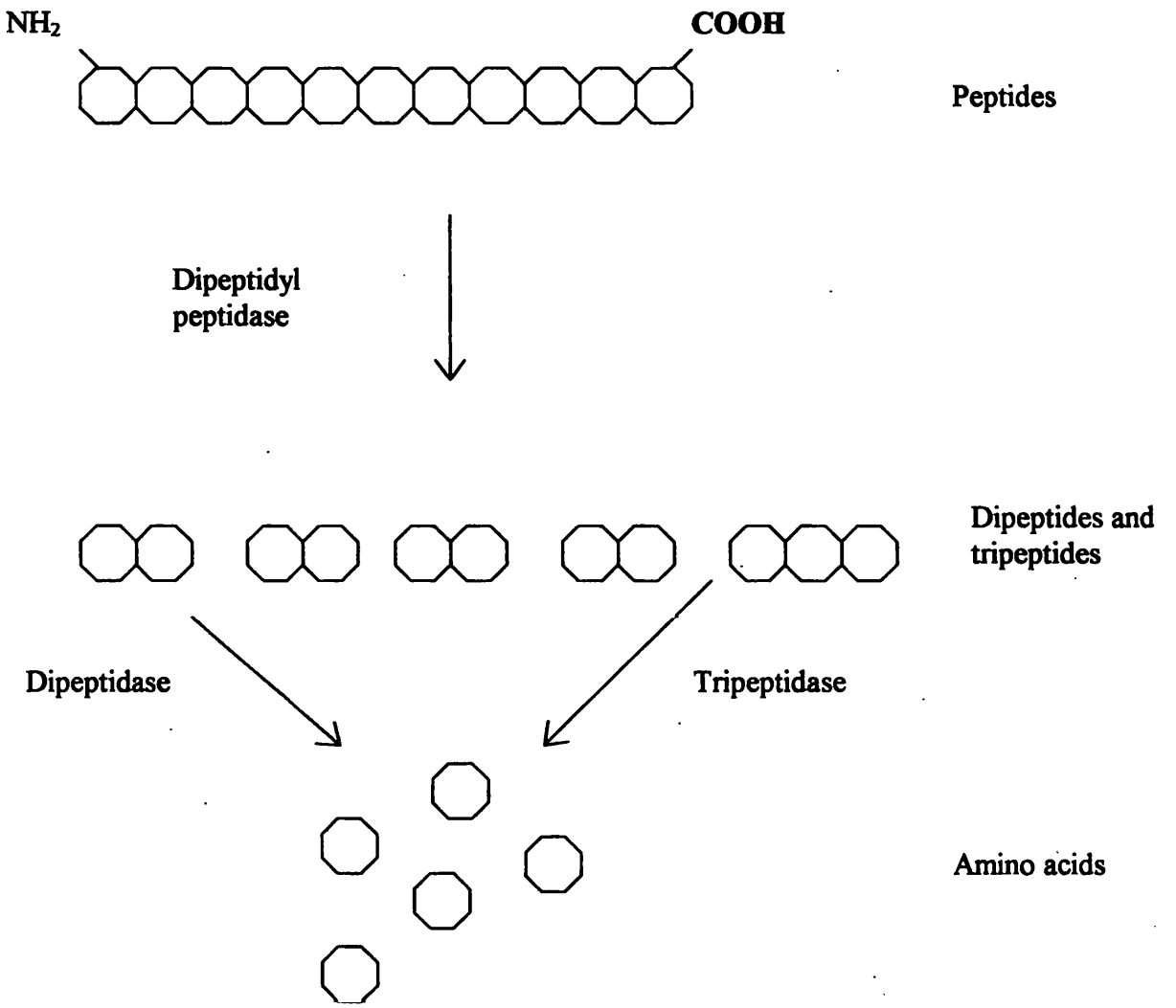


Figure 1.2. Schematic diagram of the biphasic process of peptide hydrolysis by rumen bacteria. (Wallace, 1996)

1.3.2. Effects of feed processing on protein degradability

Both physical and chemical methods are used in the processing of cereal grains and oilseeds which are included in ruminant diets. In cereal grain products, rolling and crushing methods are often used, occasionally in conjunction with heat treatment (Campling, 1991).

The methods of oil extraction used on oilseeds alter the rumen degradability of the proteins contained in the seeds (Ried, 1994), usually decreasing the degradability. The changes in protein solubility and degradability due to heat treatment may differ between different protein sources (Mir *et al.*, 1984), possibly due to differences in protein structure. Prestlokken (1999) reported that expander treatment of soya bean meal decreased effective protein degradability at all temperatures examined (130°C, 155°C and 170°C), whilst treatment of rape seed meal increased effective protein degradability at the lower temperature (130°C), but decreased it at the higher treatment temperature (170°C). Lycos and Varga (1995) reported that a decrease in particle size increased crude protein degradability in both soya and corn, and that heat treatment decreased protein degradability. Heating facilitates the Maillard reaction between sugar aldehyde groups and the free amino groups of protein to yield an amino-sugar complex which is resistant to enzymic hydrolysis (Stern *et al.*, 1994). Formaldehyde treatment of soya bean meal has also been shown to increase the resistance of the protein to ruminal degradation (Waltz and Stern, 1989) as has NaOH treatment (Nishino *et al.*, 1996).

1.4. THE RUMEN MICROBIAL POPULATION.

It is the population of microorganisms within the rumen which confers upon the ruminant animal the ability to utilise feeds which would be of little value to monogastrics, and thereby occupy an important ecological niche (Chesson and Forsberg, 1997). Microbial protein is synthesised using nitrogen mainly derived from ammonia in the rumen, and the necessary energy is derived from the fermentation of plant polysaccharides. This microbial protein is then available for digestion and absorption in the small intestine providing 0.7 - 1.0 of the animals amino acid supply (AFRC 1992), and the VFA produced during fermentation of the plant polysaccharides are absorbed across the rumen wall and provide up to 0.8 of the absorbed energy (France and Siddons, 1993).

The microbial population within the rumen is made up of three major groups, bacteria, protozoa and fungi.

1.4.1. The Rumen Bacteria.

The bacteria are the most numerous of the rumen microbes, at up to 10^{11} cells/ml being reported (Hungate, 1966). The rumen bacteria can be thought of as existing in four compartments (Czerkawski and Cheng, 1989; Cheng and McAllister, 1997);

Compartment 1 - Bacteria suspended in the liquid phase of the rumen contents. This has been commonly called "strained rumen contents". It is easily sampled and is consequently the most extensively studied part of the rumen bacterial population. The bacteria in this compartment rely on soluble substrates, therefore the size of this population is affected by dilution rate and the type of diet.

Compartment 2 - Bacteria associated with the solid phase of the rumen contents, but which can be washed out. These bacteria display both soluble polysaccharide degrading enzyme activity, and cellulolytic activity (Williams & Strachen, 1984). The function of compartment 2 has been suggested to be the transfer of microbial matter, substrates and end products between compartments 3 and 1 (Cheng and McAllister, 1997).

Compartment 3 - Bacteria which are closely associated with the solids in the rumen, and which cannot be removed by physical washing and squeezing. They may be either attached to the solid digesta, or trapped within the eroded spaces (Kudo *et al.*, 1986). They will therefore only leave the rumen when the feed particles to which they are attached are small enough. It is the population of compartment 3 which is primarily responsible for the breakdown of fibrous feed (Cheng and McAllister, 1997).

Compartment 4 - The bacteria closely associated with the rumen wall form only a small proportion of the total rumen population (approximately 1%), but they serve an important purpose. Studies of this group (Cheng & Costerton, 1980; Cheng *et al.*, 1995) have found that it has a number of functions including proteolytic digestion and recycling of the protein released from sloughed epithelial cells, and conversion of the urea diffusing into the rumen into ammonia, thereby providing the other compartments with a vital substrate for growth.

1.4.2. Rumen Protozoa.

Having originally been thought of as being of no use to the ruminant, it is now clear that the rumen protozoa have a variety of effects on the rumen environment. Defaunation has been widely used to study the role of the protozoa in the rumen, and a fairly comprehensive review of these studies was undertaken by Williams and Coleman (1997). The presence of protozoa in the rumen has been shown to increase ammonia and VFA concentration and organic matter digestibility, and to decrease the efficiency of bacterial protein synthesis and flow of nitrogen to the duodenum (Williams and Coleman, 1997). Protozoa are responsible for a significant proportion of fibre degradation (Demeyer, 1981; Orpin, 1984), and also help to maintain a stable rumen fermentation by ingesting starch and soluble sugars, preventing rapid bacterial fermentation and production of lactic acid (Mackie *et al.*, 1978).

1.4.3. Rumen Fungi.

The rumen fungi are associated mainly with the lignified plant tissues that have a long residence time in the rumen, and are therefore most numerous in animals receiving high fibre diets (Grenet and Barry, 1988; Orpin, 1989). Their role seems to be fibre digestion (Bauchop,1979), and they have been shown to possess the enzymes necessary for the degradation of both cellulose and hemicellulose (Pearce & Bauchop, 1985).

1.5. FACTORS INFLUENCING THE EFFICIENCY OF MICROBIAL GROWTH.

Microbial protein production affects the pattern of amino acids which ultimately become available to the host animal by transforming feed proteins, which have a variable amino acid composition, into microbial protein, which has a relatively constant amino acid composition (Wallace, 1994). Thus, maximising the assimilation and use of ammonia by the microbes is seen as one area in which the efficiency of N utilisation may be manipulated. However, the pattern of amino acid supply to the duodenum is also influenced by the quantity and quality of DUP in the diet (Wallace, 1994). Table 1.2. illustrates that microbial protein alone may not supply adequate essential amino acids to ensure the maximum efficiency of amino acid utilisation for productive purposes.

Table 1.2. Essential amino acid composition of lamb meat and rumen bacteria (g/100g amino acids). (Adapted from Wallace, 1994).

Amino acid	Lamb meat	Mixed bacteria	Bacterial amino acids as % of lamb amino acids
Histidine	3.3	2.1	64
Isoleucine	4.7	5.7	121
Leucine	7.3	7.6	104
Lysine	9.9	8.5	86
Methionine	2.6	2.4	92
Phenylalanine	3.9	4.9	126
Threonine	4.7	5.4	115
Tryptophan	1.3	1.3	100
Valine	4.9	6.0	122

It is generally accepted that lysine, methionine and histidine are the most limiting amino acids for ruminants, and it may be possible to correct this imbalance by feeding an undegradable protein source which is high in these amino acids, or by feeding protected forms of the individual amino acids (Wallace, 1994).

The efficiency of microbial growth, and therefore the daily microbial yield are affected by a number of factors including nitrogen source, energy source, concentrate to forage ratio, level of intake and pattern of feeding.

1.5.1. Energy source.

The availability of energy is usually the most limiting factor for microbial growth (Hoover and Stokes, 1991; Febel and Fekete, 1996). Microbes derive their energy from the fermentation of plant carbohydrates, both structural and non structural. Non structural carbohydrates are rapidly fermented due to their soluble nature whilst structural carbohydrates are fermented more slowly (Leedle *et al.*, 1986). The VFA produced, which are absorbed and utilised by the animal, are effectively waste products to the microbes. The valuable product is ATP which the microbes use for maintenance and the production of new microbial cells. Bauchop and Elsden (1960) introduced the concept of Y_{ATP} which is defined as the quantity of microbial dry matter (g) which can be synthesised from one mole of ATP, and was assumed to have a constant value of 10.5. However it has subsequently been demonstrated that values can range from 4.6 to 20.9 (Southamer and Bettenhausen, 1973; Russel and Wallace, 1989). Values are affected by the source of energy or nitrogen available to the microbes (Stouthammer, 1977, 1973; Sniffen and Robinson, 1987) with the highest growth rate being achieved using monosaccharides as an energy source, and the lowest growth rate obtained when cellulose is used as an energy source (Sniffen and Robinson, 1987). ATP production is usually calculated from the production of VFA, however the absolute yields of ATP are not known for propionate (3,4 or 6 moles) and

butyrate (2 or 3 moles) (Baldwin *et al*, 1970; Hobson and Summers, 1972; Thauer *et al*, 1977). In practice microbial efficiency is expressed on the basis of g microbial N per kg OM apparently digested in the rumen (ie. the OM disappearing between the mouth and the abomasum or duodenum), or g microbial N per kg OM truly digested in the rumen (ie. the OM disappearing plus the OM of the synthesised microbes) (ARC, 1980). The mean value adopted by the Agricultural Research Council (ARC 1984) for the efficiency of microbial N synthesis was 32g/kgOMADR. However, for silage fed animals, this value is consistently lower, tending to be in the range 25- 30g/kgOMADR (ARC 1984).

The maintenance functions of the rumen microbes have not been precisely defined, but functions such as maintenance of the ion balance across the cell membranes, motility and macromolecule transport may be important (Tempest and Neijssel, 1984; Russell and Wallace, 1989). If microbial growth is limited by a deficiency in supply of some other growth factor, nitrogen for example, energy sources are still consumed at a rapid rate (Neijssel and Tempest, 1976). This results in a reduction in production of microbial biomass, and an increase in heat production. This has been termed "energy spilling" or "uncoupling". Russell (1986) found that energy sufficient cultures produced more heat than energy limited cultures. Heat production increased rapidly on pulse dosing with glucose and decreased rapidly once glucose was depleted, indicating that the change could not be caused by changes in protein synthesis. It is also possible that this heat production is caused by ion recycling. According to the model of Mitchell *et al.* (1961) energy is utilised to translocate protons outwards, through the membrane to produce a proton motive force for transport of potassium, magnesium and ammonium into the cell in preparation for future rapid growth. If some of these ions leak back out of the cell there is continued heat production.

Several studies have been carried out investigating the effect of different energy sources on rumen fermentation and the efficiency of microbial growth. Many of these studies have concentrated on methods of increasing the efficiency of microbial N synthesis on silage based diets by giving energy supplements in various forms. Chamberlain *et al.* (1985) found that when silage fed sheep received supplements of various carbohydrates, the rumen

ammonia concentration decreased in all cases, although the decrease seen with starch was not as pronounced as that seen with glucose and sucrose. These results were confirmed in a later experiment (Chamberlain *et al.* 1993) in which sugar supplements caused a greater reduction in ruminal ammonia than did starch. At the same time as the rumen ammonia decreased, urinary excretion of purine derivatives increased significantly in all cases except that of starch. Calculated values for the supply of microbial N to the duodenum showed an increase over silage alone for all supplements, but the increase shown with sucrose supplementation was three times greater than that shown in the case of starch supplementation. This study indicated a superior microbial utilisation of silage N with sugar supplements than with starch. However, in practice, it is difficult to feed sugar supplements to ruminants without a resultant drop in ruminal pH, and consequent reduction in fibre digestion (Chamberlain *et al.*, 1993).

Stern *et al.* (1978) investigated the effect of changing the proportion of starch to cellulose in an iso-nitrogenous diet, on microbial protein synthesis in continuous culture and reported that increasing the starch: cellulose ratio resulted in decreases in ammonia concentration, increases in microbial protein synthesis, and increases in Y_{ATP}. Offer *et al.* (1978) gave silage fed sheep supplements of either wheat starch or paper or both, and found that at high levels of supplementation, amino acid N entering the duodenum was increased over the basal diet for all supplements. Over a three day period, the flow of amino acid N increased by 5.3g on the paper supplement and 23g on the wheat starch supplement, but the greatest increase (25.7g) was seen when a mixture of the two was fed. When expressed as microbial total amino acids passing to the duodenum per MJ energy apparently disappearing between the mouth and the duodenum, the values for the mixture of paper and starch were significantly higher than for starch or paper alone. This lead to the conclusion that the energy from a mixture of the two supplements was used more efficiently for the synthesis of microbial protein (Offer *et al.*, 1978).

Rooke *et al.* (1985) added barley to a basal diet of silage resulting in a reduction of rumen ammonia in Jersey heifers from 82mg/l to 45mg/l. This corresponded with an increase in the

efficiency of microbial N synthesis (gN/kgOMADR) from 24.3 to 30.1. Similarly Thomas *et al.* (1980) found that supplementing spring cut silage with barley caused an increase in both the quantity of bacterial N passing to the duodenum (5.91 to 8.55g bacterial N/d), and the efficiency of microbial N synthesis (22.72 to 25.76 gN/kgOMADR).

It would seem then that adding a concentrate containing a readily available source of energy to a forage based diet results in an improvement in microbial efficiency. However it is not only the source of energy which will have an effect, the level of inclusion is also important. Alteration of the forage to concentrate ratio in the diet has been seen in a number of studies to cause alterations in the efficiency of microbial production. It appears that the optimum inclusion rate of concentrate in diets for sheep is about 30% DM (Chamberlain and Thomas, 1979; Mathers and Miller 1981). Inclusion rates above and below this point caused decreases in the amount of microbial protein synthesised (g/kgOMADR). Cole *et al.* (1976) found that increasing concentrate supply to ruminants already on high concentrate diets (ie above 66% inclusion) caused a decrease in microbial efficiency. However, Archimede *et al.* (1996) found no difference in microbial efficiency when concentrate inclusion was increased from 30% to 60% in goats, a result in agreement with Merchen *et al.* (1986) who found no effect on microbial efficiency when concentrate level was increased from 25% to 75% in sheep.

1.5.2. Nitrogen source

Rumen microbes can synthesise protein using N from various sources. Both cellulolytic and amylolytic bacteria use ammonia, amino acids, peptides and dietary non-protein nitrogen (NPN) (Russell *et al.*, 1992; Carro and Miller, 1999). The major source of these nitrogenous compounds is feed protein, with a small contribution being made from endogenous sources and recycled N. It has been shown that depending on diet, 0.4-0.6 of bacterial N is derived from ammonia (Leng and Nolan, 1984). It is probable that ammonia

enters microbial cells by passive diffusion of the un-ionised form (Nolan, 1993), and is assimilated primarily by the action of NAD-linked glutamate dehydrogenase (Wallace *et al.*, 1997). Amino transferases in the microbial cells then allow transfer of the NH_3 groups from glutamate to form other amino acids in the intracellular pool (Wallace *et al.*, 1997).

There is some disagreement as to the optimum ammonia concentration for microbial synthesis. Satter and Slyter (1974) found no benefit in increasing rumen ammonia concentration above levels of 50mg/l. However Mehrez *et al.* (1977) reported that the minimum concentration required for maximum microbial synthesis was as high as 235mg/l. Hespell and Bryant (1979) established that when diets high in rapidly fermentable carbohydrate are fed, a higher concentration of ammonia is required to maximise microbial synthesis. These variations in optimum level may be explained by the bacteria having more than one NH_3 fixing mechanism, allowing them to utilise differing levels of NH_3 with equal efficiency (Harrison and McAllen, 1979). The synthesis of bacterial amino acids from NH_3 appears to proceed via two major pathways, the glutamate dehydrogenase pathway, which dominates when ammonia concentrations are above 1mM and does not require an input of ATP, and the glutamine synthetase/glutamate synthetase pathway which operates under low NH_3 concentrations and requires the input of ATP (Hespell, 1985).

In vitro studies comparing different forms of N supplement, and their effect on ruminal fermentation and crude protein digestion have found that the more soluble the N source, the higher the rumen NH_3 concentration. However provided the rumen NH_3 concentration does not fall below the recommended 50mg/l (Satter and Slyter, 1974), bacterial N flow and the efficiency of microbial protein synthesis are not affected (Bas *et al.*, 1989; Calsamiglia *et al.*, 1995).

There is clear evidence that the rumen bacteria are capable of utilising amino acids and peptides as well as ammonia (Maeng and Baldwin, 1976; Maeng *et al.*, 1976; Argyle and Baldwin, 1989; Griswold *et al.*, 1996; Chikunya *et al.*, 1996; Carro and Millar, 1999). Some of these *in vitro* studies indicate that certain bacteria have a requirement for amino acids and peptides in order to achieve maximal growth. The assumption that the inclusion of true protein increases bacterial growth is supported by the work of Argyle and Baldwin (1989), who reported that little or no growth occurred when urea alone was added to cultures of mixed rumen bacteria. Addition of a combination of peptides and amino acids quadrupled growth, and the addition of peptides alone caused a greater increase in growth than the equivalent amount of free amino acids. Cotta and Russell (1982) carried out a series of experiments designed to ascertain the effects of peptides and amino acids on the efficiency of microbial growth in pure culture. They found that reducing the supply of amino acids caused decreases in the yields of microbial protein, the greatest reductions were found at levels below 0.062g/l. Although the total yield of microbial protein was always highest at the highest amino acid concentration, the most efficient use of amino acids occurred at concentrations of 0.031g/l. Therefore the level of amino acid supply needed to maximise microbial yield is not the same as that required to maximise the efficiency of amino acid use. The hypothesis that amino acids and peptides are required to maximise microbial growth is supported by the intraruminal infusion work of Rooke and Armstrong (1989), when it was found that infusion of casein into the rumen of silage fed cattle increased the quantity of microbial N entering the duodenum to 126g/d as compared with 108g/d when urea was infused. The efficiency of microbial N synthesis was also higher on casein infusion than on urea infusion (34gN/kgOMADR and 29gN/kgOMADR respectively). Feeding soya bean meal had intermediate effect on both parameters. Rooke *et al.* (1989) also reported that infusion of casein and sucrose into the rumen of silage fed cows produced an increase in the quantity of microbial protein synthesised, whereas infusion of urea and sucrose did not. This suggests that it was the products of casein hydrolysis which were causing the increased microbial protein synthesis, rather than simply the supply of supplementary N. In practical feeding situations this means that inclusion of ruminally degradable protein sources such as soya bean meal in the diet will increase the supply of amino acids and peptides to the

on the supply of amino acids to the peripheral tissues, with consequent penalties on protein gain.

Urea recycling is a characteristic unique to the ruminant, and serves to supplement low protein diets. From 0.23 to 0.92 of plasma urea may be recycled to the digestive tract (Kennedy and Milligan, 1980) either via saliva or via direct transfer through the rumen wall. The amount of salivary N entering the rumen is influenced by the physical form of the diet, and the amount of rumination taking place and for cattle and sheep on forage diets has been shown to constitute 0.15 to 0.5 of urea transfer to the rumen (Kennedy and Milligan, 1980). The concentration of urea in the saliva is directly correlated with blood concentration, and therefore with the level of rumen degradable N in the diet (Egan *et al.*, 1986). Transfer of urea across the rumen wall is by diffusion (Houpt, 1970; Egan *et al.*, 1986) with the urea diffusing into the mucosa from the blood stream being hydrolysed to ammonia by the ureases of the population of rumen bacteria which are closely associated with the rumen wall. The quantity of urea entering the rumen by this route is dependant on plasma urea concentration and the rate of blood flow through the rumen wall (Nolan, 1993) and is decreased in times of high rumen ammonia concentration due to a decreased ammonia diffusion gradient, or inhibition of the bacterial urease (Owens and Bergen, 1983). Overall the recycling of urea to the rumen is positively correlated with the rate of apparent digestion of OM in the rumen, and with plasma urea concentration (Kennedy and Milligan, (1980).The maximum estimate of N recycling to the rumen for sheep is 6g/d (Houpt, 1970). If the diet is very low in protein, the quantity of nitrogen being supplied to the rumen via N recycling may be greater than the quantity being absorbed from the rumen as ammonia. This recycled nitrogen can then be used for microbial protein synthesis, resulting in the amount of protein reaching the duodenum exceeding that supplied in the diet (Egan *et al.*, 1986). This process of N recycling carries an energy cost to the animal, and it has been reported

that the higher the NH_3 load on the liver, the higher the liver O_2 consumption (Lobley *et al.*, 1995).

The ammonia N which is neither assimilated by the rumen microbes, nor recycled to the rumen is excreted in the urine and constitutes a 'waste' of dietary protein N (Morrison and Mackie, 1996). It has been estimated that in the USA this 'waste' N may account for up to 20% of protein fed, the cost of which may approach five billion dollars (Russell, 1993). Thus minimising this loss of N would be of both environmental and economic benefit.

1.7.2.2. Metabolism of purines and pyrimidines

Absorbed purines and pyrimidines are, for the most part, catabolised into their metabolic end products, hypoxanthine, xanthine, uric acid and allantoin (Armstrong and Hutton, 1975; Chen and Gomez, 1992). The site of catabolism differs between cattle and sheep, with cattle converting most of the absorbed bases into uric acid in the intestinal mucosa, where as in sheep the bases enter the liver unchanged and are available for either catabolism or incorporation into tissue nucleic acids. The products of purine and pyrimidine catabolism are excreted in the urine (Chen and Gomez, 1992).

1.7.2.3. Metabolism of amino acids

Not all the amino acids available for absorption from the small intestine appear in the portal blood (Seal and Reynolds, 1993). Possible reasons for the disappearance of these amino acids are the high rate of protein turnover within the gastrointestinal tract, and the use of amino acids as energy sources (Lobley *et al.*, 1980). Gastrointestinal protein synthesis can

account for 38-46% of whole body protein synthesis in cattle (Lobley *et al.* 1980), and rates of synthesis vary with diet and physiological state (Seal and Reynolds, 1993). It has been reported that intraruminal infusion of propionate increases the level of circulating amino acids, possibly due to a decreased utilisation of amino acids by the intestinal tissues (Seal and Parker, 1991; Seal *et al.*, 1993).

Amino acids absorbed across the portal drained viscera are removed by the liver with the exception of the branched chain amino acids (Armstrong and Hutton, 1975; Seal and Reynolds, 1993). Most of the amino acids are utilised in the synthesis of export proteins (Lindsay, 1980; Lobley *et al.*, 1998), and the remainder catabolised. Most plasma proteins, with the exception of γ-globulin, are synthesised in the liver (Armstrong and Hutton, 1975). The liver itself accounts for only 10% or less of whole body protein synthesis in ruminants, but weight for weight is one of the most metabolically active organs accounting for 25% of total energy expenditure (Lobley, 1994). Bergmann *et al.* (1978, 1986) carried out a series of experiments investigating the amino acid metabolism of the ruminant liver. These studies identified that free amino acids are involved in the inter-organ exchange of carbon and nitrogen. The extrasplanchnic tissues were net contributors of the glucogenic amino acids alanine, glycine and serine to fulfill the liver's requirement for substrates for gluconeogenesis. The liver released glutamate, which was removed by the hindlimb tissues, and removed glutamine, which was released by the hind limb tissues. A similar relationship was seen between the liver and kidneys, in which the liver removed arginine and released citrulline and ornithine, with the opposite being true of the kidneys (Reynolds, 1992). This inter-organ cycling of amino acids allows the transfer of ammonia and carbon to the liver for urea and glucose synthesis. The metabolism of amino acids by the ruminant liver may be affected by diet (Reynolds, 1992). Reynolds *et al.* (1991) reported that in growing cattle,

increased intake of an alfalfa/concentrate diet increased net absorption of amino acids across the portal drained viscera, and increased hepatic uptake of amino acids. However the increase in hepatic uptake did not match the increase in absorption, thus there was an increased splanchnic release of amino acids.

Amino acids not utilised in protein synthesis, are catabolised, yielding ammonia and carbon skeletons. The ammonia metabolism of the liver has previously been discussed. The carbon skeletons are utilised as an energy source and provide a major source of carbon for glucose synthesis (Armstrong and Hutton, 1975). Amino acids may be classified as being glucogenic or ketogenic (Stryer, 1988). Ketogenic amino acids are those which are degraded to give ketone bodies (acetyl CoA or acetoacetyl Co A), and glucogenic amino acids are those which are degraded to give pyruvate, α-ketoglutarate, succinyl CoA, fumarate or oxaloacetate. These metabolites can contribute to net synthesis of glucose via the TCA cycle (Figure 1.3.) The contribution of glucogenic amino acids to total glucose production in the animal will vary depending on the nutritional and physiological status of the animal. Studies with mature cattle fed near maintenance showed that glucogenic amino acids contributed 0.33 to 0.49 of total glucose produced (Maltby *et al.*, 1993; Reynolds *et al.*, 1994). Similar studies in lactacting dairy cows fed *ad libitum* found that glucogenic amino acids contributed up to 0.55 of hepatic glucose output. The glucose thus generated can then be metabolised via the pentose phosphate pathway producing NADPH and glycerol-3-phosphate, both of which are essential in the anabolism of acetate to fat (Vernon, 1992).

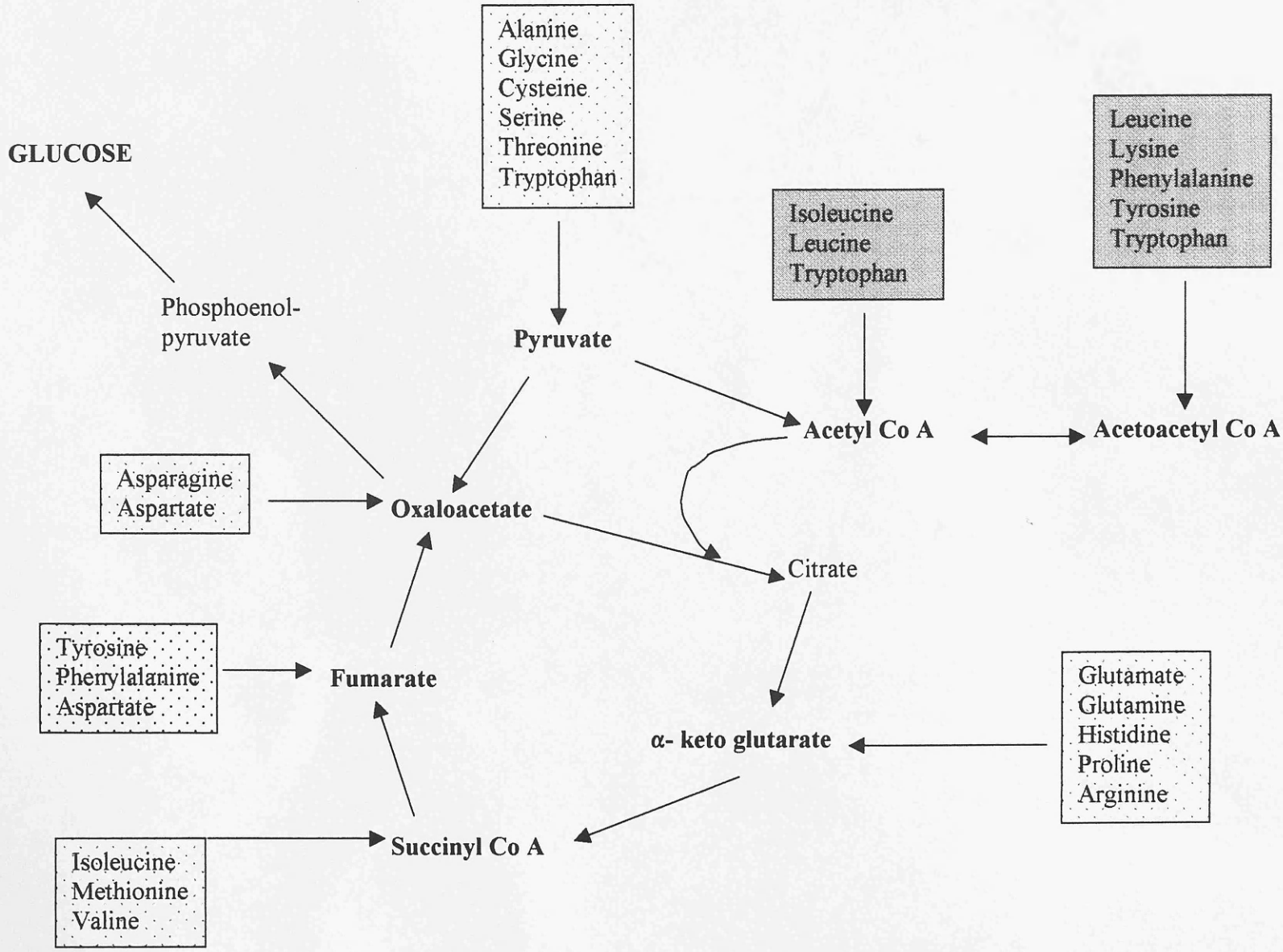


Figure 1.3. Fates of the carbon skeletons of the amino acids. From *Biochemistry*. L Stryer, 1988.

= Glucogenic AAs = Ketogenic AAs

1.8. CONTROL OF ANIMAL GROWTH AND DEVELOPMENT.

The growth of an animal is a function of its genetic capacity to grow, the nutritional status of the animal and aspects of its environment (Beever *et al.*, 1993). The overall regulator of growth is considered to be the endocrine system, with growth hormone (GH) playing a key role, although it is accepted that the GH axis itself is under direct nutritional control (Bass *et al.*, 1992).

The overall composition of the animal body is influenced by four main factors: maturity, breed, sex and nutrition (Gill and Oldham, 1993).

1.8.1. Maturity

Carcass fat content increases steadily in the period from birth to maturity (Searle *et al.*, 1972; Arana *et al.*, 1998). Weight gain is made up of differing proportions of fat protein and water depending on the liveweight of the animal. Increase of any part of the animal was first described by Huxley's allometric equation proposed in 1932 where;

$$y = bx^a$$

$$\text{Or } y = \log b + a \log x$$

Where y = weight of the part (water, protein, ether extract or ash)

x = weight of the whole (whole body, empty body or carcass)

a = growth coefficient

b = constant

The growth coefficient a is a measure of the rate of growth of the part relative to the whole. A coefficient greater that 1.0, indicates that part is increasing at a greater rate than the whole, and therefore increasing in proportion within the whole. From Figure 1.4. it can be seen that as the live weight of the animal increases, so the contribution of fat to the weight gain increases.

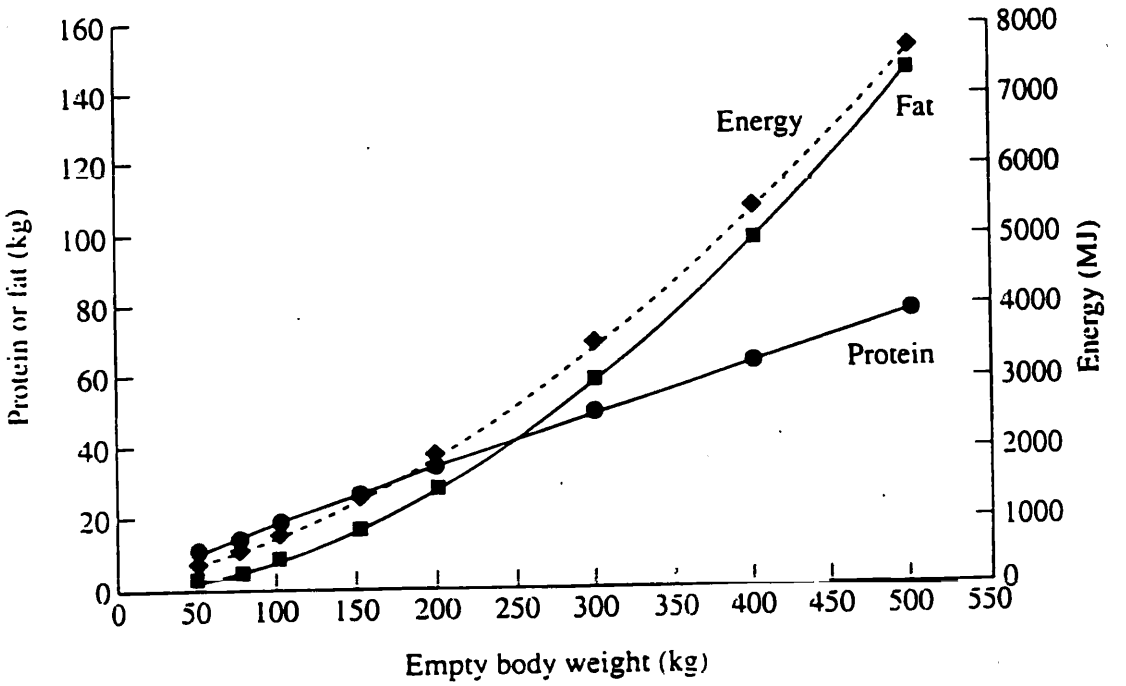


Figure 1.4. Growth of protein, fat and energy in cattle. (From: Animal Nutrition, Fifth Edition. 1995. P. McDonald, R. A. Edwards, J. F. D. Greenhalgh and C. A. Morgan.)

In more recent years, many equations have been proposed to predict growth and composition of gain (Gill and Oldham, 1993) almost all of which estimate growth and composition of gain as a function of body weight. Friggens *et al.* (1997) and Zygoyiannis *et al.* (1997) confirmed that the proportions of lean tissue in the carcass decreased, and that of fat increased with increasing carcass weight across a range of breeds.

1.8.2. Breed effects on growth

Breed effects on growth and carcass composition are generally related to differences in mature weight (Gill and Oldham, 1993). At a given live weight, animals of a larger mature size will be at a lower proportion of maturity than those of a smaller mature size, and will therefore have a higher proportion of protein in the body, and in any live weight gain (Gill and Oldham, 1993). In the case of cattle, AFRC (1993) recommends the classification of beef breeds into early, medium or late maturing, and the use of a correction factor to account for the lower energy content of live weight gain in the late maturing breeds. However no such correction is recommended for sheep, with the exception of the exclusion of Merino type breeds. Taylor *et al.* (1989a, b, c) reported significant differences between sheep breeds in proportions of carcass muscle, fat and bone. In readily fattened breeds (Welsh Mountain, Southdown, Wiltshire Horn and Oxford Down) the rate of increase in the proportion of fat in the carcass was three times that of the less readily fattened breeds (Soay, Jacob and Finnish Landrace), even allowing for genetic size scaling. However throughout this series of experiments, Taylor *et al.* (1989a, b, c) point out that if small, deviant breeds are excluded from the analysis (eg. Soay) then most interbreed differences disappear after genetic size scaling (Thonney *et al.*, 1987a, b and c; Taylor *et al.*, 1989a, b and c).

1.8.3. Sex effects on growth and carcass composition.

AFRC (1993) recommend classification of both cattle and sheep into males, castrates and females when calculating the energy content of live weight gain. This reflects the fact that entire males grow faster, with a lower proportion of fat in the gain than castrates or females (Galbraith and Topps, 1981). Thonney *et al.* (1987a) reported that across several breeds of sheep, there was a tendency for males to consume more food and use it less efficiently than females.

1.8.4. Effects of nutrition on growth and carcass composition.

As discussed in sections 1.8.2 and 1.8.3., the breed and sex of an animal determine its potential for lean and fat deposition. However the ability, or otherwise of the animal to achieve its inherent growth target will be determined by the supply of nutrients from the diet. The availability of nutrients plays a role in determining the partitioning of those nutrients to the various body tissues and processes (Van der Walt, 1985).

For ruminants the net conversion of feed N to body protein is an inefficient process (Lobley, 1992). The main substrates for tissue protein synthesis in ruminant animals are amino acids absorbed from the intestine following hydrolysis of microbial or feed protein (Armstrong and Hutton, 1975). The quantity and pattern of amino acids available for absorption in the duodenum is influenced by the quantity and quality of the diet fed to the animal (Beever *et al.*, 1993). The principal substrates for body fat synthesis are acetate arising from rumen fermentation and glucose arising from gluconeogenesis in the liver, therefore manipulation

of rumen fermentation may also be used to alter supply of lipogenic precursors (MacRae and Lobley, 1984).

1.8.4.1. Protein – energy interrelationships.

MacRae and Lobley (1984) suggested that it is naïve to think of nutrients as being either energy or protein yielding, as nutrients can be diverted and interchanged to supply the optimum pattern needed to meet the requirements for a particular physiological state (growth, pregnancy or lactation). Central to this process is the tricarboxylic acid (TCA) cycle, which is the main pathway for most energy yielding oxidative processes, and provides key carbon skeletons for the production of fat, protein and glycogenic precursors (MacRae and Lobley 1984). The interrelationships between protein and energy yielding substrates can have large effects on the overall pattern of nutrient use. For example, changing the input of protein can influence animal performance both by changing the overall plane of nutrition through effects on feed digestibility and intake, and secondly by changing the pattern and efficiency of use of absorbed nutrients (Oldham, 1984). The study of efficiency of nutrient utilisation can be approached from two directions; 1) the effect of energy yielding nutrients on the efficiency of protein utilisation, 2) the effects of protein on the utilisation of energy. The maximum efficiency of protein utilisation has been found to occur on diets which are so protein limited as to be incompatible with optimum performance (Satter and Roffler 1975; Gordon and McMurray, 1979). Therefore most studies have focused on the effect of supplementation with protein on energy utilisation and animal performance.

A study by Storm *et al.* (1983) illustrated that there is a close relationship between energy availability and protein deposition in lambs. Where energy availability was approximately twice maintenance, N retention increased linearly with increasing N infusion into the abomasum. Where energy availability was equal to maintenance there was no increase in N retention with increasing N infusion (Storm *et al.*, 1983). Figure 1.5. illustrates that production increases with increasing N supply, but the maximum response to N is limited by energy availability.

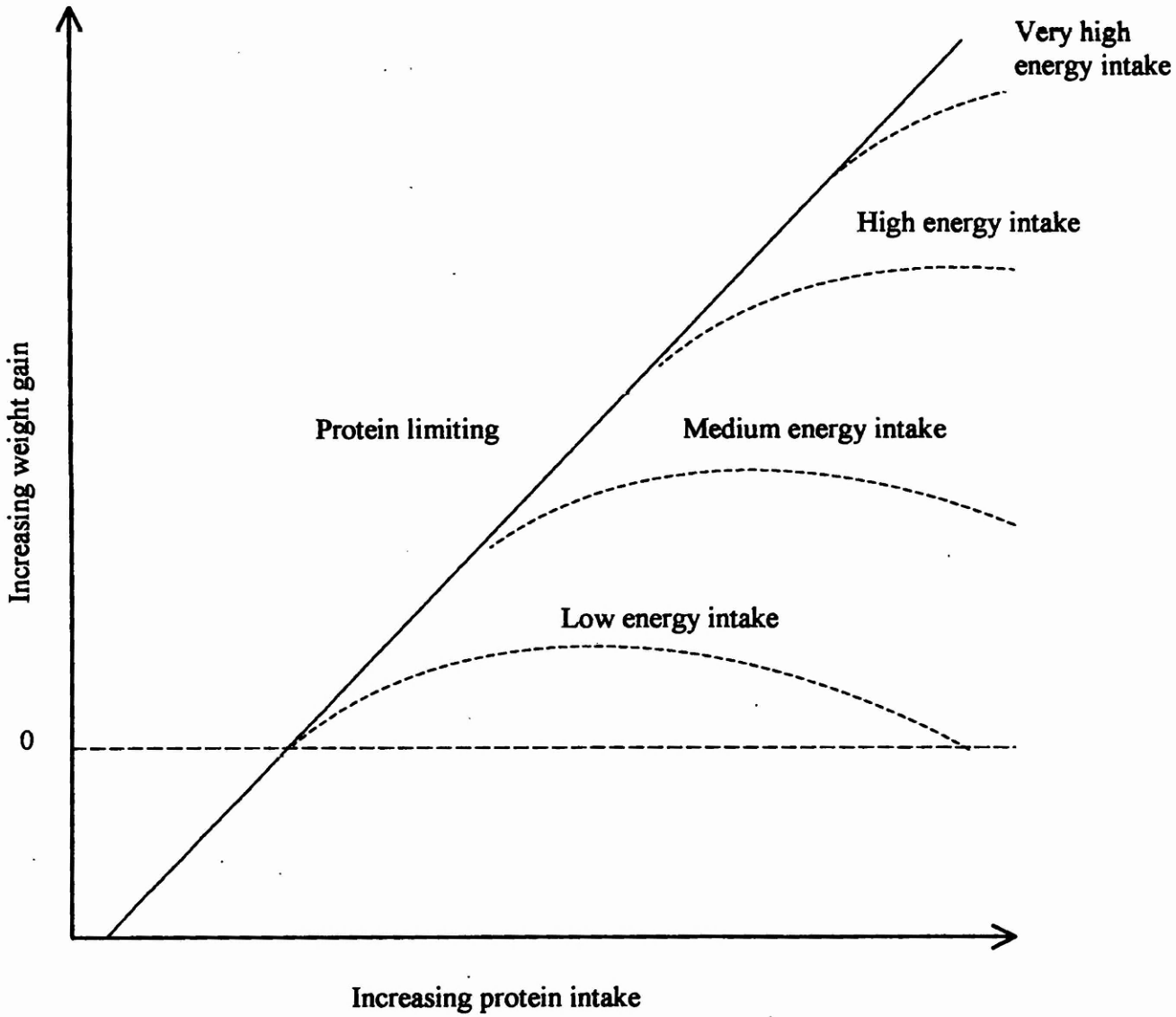


Figure 1.5. The response of growing ruminants to the combined effects of energy and protein intake. (Adapted from Figure 4.7 in The Nutrient requirements of Ruminant Livestock. ARC 1980)

The hypothesis that protein synthesis is an energy consuming process is supported by the data of Reeds *et al.* (1980) who reported a correlation between heat production and protein synthesis in pigs. MacRae and Lobley (1984) found a similar relationship in finishing beef steers, where each MJ increment in heat production was associated with 40g of protein synthesis. Therefore it could be postulated that if energy supply were to be limited, protein synthesis would decline (MacRae and Lobley, 1984).

Cellular processes other than protein synthesis are also energy dependant. One of the most studied is the maintenance of Na^+ and K^+ gradients across the plasma membrane catalysed by Na^+, K^+ ATPase, which is also involved in the transport of other metabolites (Gregg and Milligan, 1982a, b, c). Estimates of the contribution of this process to the total requirement for aerobic respiration vary from 40% to 5% depending on the supply of nutrients, as an increased nutrient supply will lead to an increased metabolic load (MacRae and Lobley, 1984).

It has been shown that N retention decreases with increasing molar proportions of acetate and decreasing molar proportions of propionate (Ørskov *et al.*, 1979). It is likely that as molar proportion of propionate decreased, more amino acids were catabolised to support acetate metabolism. If animals on diets producing a high acetate:propionate ratio are supplied with additional amino acids, the efficiency of ME utilisation is increased (i.e. heat production is decreased) (MacRae *et al.*, 1985), probably due to an increased supply of NADPH and glycerol-3-phosphate from the catabolism of glucogenic amino acids enabling more efficient utilisation of acetate. It has long been recognised that a lack of NADPH and glycerol-3-phosphate may limit the conversion of acetate to long chain fatty acids (Armstrong, 1965), and the crucial role of glucose from either propionate or glucogenic amino acids in providing these compounds has since been revealed (Gill *et al.*, 1984; Black *et al.*, 1987).

1.8.5. Hormonal control of growth

The main endocrine system involved in the control of post natal growth is the growth hormone (GH) axis (Gluckman *et al.*, 1983) which is sensitive to nutritional changes (Bass *et al.*, 1992).

1.8.5.1. The Growth Hormone Axis.

Growth hormone is released from the pituitary gland in a pulsatile manner (Klindt and Stone, 1984; Dubreuil *et al.*, 1988). Its release is stimulated by Growth Hormone Releasing Factor (GRF) and inhibited by Somatostatin, both of which are released from the hypothalamus (Frohman *et al.*, 1990). This is the first stage at which the nutritional status of the animal can influence GH secretion. Both free fatty acids (FFA) and glucose have been shown to stimulate somatostatin secretion, which in turn reduces the levels of circulating growth hormone (Imaki *et al.*, 1986; Spencer *et al.*, 1990). Growth hormone enters the circulation where it becomes associated with growth hormone binding proteins (GHBP). Protein bound growth hormone has an increased half life, and it has been shown that a high plane of nutrition causes an increase in the circulating levels of binding proteins, and therefore in the half life of growth hormone (Bauman *et al.*, 1987). The main site of action of growth hormone is the liver, where it binds to growth hormone receptors (Barnard *et al.*, 1988). The liver then produces insulin like growth factor (IGF-1) which mediates the protein anabolic effects of growth hormone (Daughaday *et al.*, 1972; Dawson *et al.*, 1998). This appears to be the second main point at which nutritional influences come into play. Restricted feeding causes a decrease in the number of growth hormone receptors (Maes *et al.*, 1983, 1986; Breier *et al*, 1988a; Breier, 1999), and in the GH binding capacity of hepatic GH receptors (Breier, 1999) and therefore in the secretion of IGF-1. This goes some way to explaining the perceived paradox that a low nutritional status causes an increase in growth hormone secretion and a decrease in IGF –1 (Breier and Sauerwein,

1995). As a low plane of nutrition causes a decrease in both GHBP and GH receptors, resulting in a decrease in IGF-1 levels, the level of circulating GH must be increased in order to maintain basal metabolism (Bauman and Currie, 1980; Bines and Hart, 1982).

The action of IGF-1 on the tissues is mediated by both somatomedin inhibitors and IGF receptors (Bass *et al.*, 1992). It appears that starvation causes an increase in the level of somatomedin inhibitors, thereby inhibiting the action of IGF-1 (Phillips, 1986). However there is very little evidence of nutritional effects on IGF receptors (Bass *et al.*, 1992). The action of IGF-1 on tissues, particularly muscle has been shown to cause an increase in protein synthesis, and a decrease in degradation, resulting in an increase in protein deposition (Reeds and Davis, 1992). Growth hormone also has the effect of inhibiting lipogenesis in ruminant adipose tissue (Vernon and Flint, 1989), and antagonises the ability of insulin to stimulate lipogenesis (Vernon *et al.*, 1991). The effect of GH on adipocytes is thought to be mediated by GH itself rather than via IGF-1 as is the case with protein metabolism (Dawson *et al.* 1998) as adipocytes do not posess IGF-1 receptors (Vernon and Flint, 1989). Dawson *et al.* (1998) reported that administration of exogenous GH increased plasma palmitate flux rates and plasma non-esterified fatty acid (NEFA) concentrations in steers across a range of nutritional states. However the response to GH, in terms of palmitate flux rates and plasma NEFA, was suppressed at the higher levels of intake, possibly due to a reduction in the number of GH receptors in the adipose tissue. The administration of exogenous GH has resulted in an average increase in growth rate of 12% (Buttery and Dawson, 1990), and is associated with an increase in protein deposition and a decrease in fat deposition (Enright, 1989).

Within the GH axis there are two negative feedback mechanisms (Bass *et al.*, 1992). IGF-1 has a direct effect on the secretion of GH, acting on the pituitary, but this mechanism is too slow to be a major short term control (Abe *et al.*, 1983). GH itself has a much more rapid effect, elevated levels causing an increase in the secretion of somatostatin, and therefore inhibiting the secretion of GH (Tannenbaum, 1981).

1.8.5.2. Other hormones affecting growth.

Insulin.

Insulin is known to be involved in the homeostatic regulation of nutrient partitioning, ie. the maintenance of a constant internal environment in the face of external changes, specifically by stimulating the uptake by tissues of glucose in the absorptive state immediately following a meal (Bauman, 1985). Insulin stimulates uptake and utilization of glucose by many peripheral tissues, inhibits gluconeogenesis and glycogenolysis in the liver, stimulates the uptake of amino acids and their incorporation into protein whilst inhibiting proteolysis, and stimulates lipogenesis and inhibits lipolysis (McDowell, 1983). Insulin is known to stimulate protein synthesis in muscle tissue by stimulating translational efficiency and amino acid transport (Reeds and Davis, 1992). It is also known that insulin decreases hepatic proteolysis (Mortimore and Surmacz, 1983), and it is generally held that the effect on muscle proteolysis would be similar, however there is little direct evidence to support this assumption (Reeds and Davis, 1992). Therefore the overall effect of insulin is to increase protein deposition.

Insulin also has an effect on lipid metabolism. Scollan and Jessop (1995) reported that increasing the insulin concentration in the incubation medium caused increased acetate oxidation and incorporation into lipid in ovine perirenal adipocytes *in vitro*, which is broadly in agreement with other studies, although the level at which maximum response was obtained is not clear (Etherton and Evock, 1986). The stimulation of lipogenesis by insulin is mediated by the activation of acetyl co-A carboxylase (Vernon, 1992). Incubation of ruminant adipose tissue with insulin for 24 hours results in an increased rate of lipogenesis (Vernon and Sasaki, 1991), but the action of insulin on lipogenesis is antagonised by GH (Vernon *et al.*, 1991). Insulin also acts to decrease the rate of lipolysis through two distinct mechanisms, one through decreasing the cellular concentration of cyclic-AMP which plays a role in the activation of hormone-sensitive lipase, and one which is cyclic-AMP independent, but also results in decreased activity of hormone-sensitive lipase (Vernon, 1992).

Glucagon

Glucagon acts in opposition to insulin, and its primary effects are to increases hepatic glucose output by increasing gluconeogenesis and glycogenolysis (McDowell, 1983). Thus the ratio of insulin : glucagon allows maintenance of glucose homeostasis.

Thyroid hormones

The thyroid hormones (thyroxine (T_4) and 3,5,3'-triiodothyronine (T_3)) have been shown to affect both protein synthesis and degradation, with increasing levels increasing both processes (Reeds and Davies, 1992). Overall, hyperthyroidism results in a decreased skeletal mass, as the increase in protein breakdown is greater that the increase in protein synthesis (Buttery, 1983). Thyroidectomy has been shown to result in severe growth retardation, which can be restored to near normal by administration of exogenous hormone (Lawrence and Fowler, 1997) Thyroid hormones are transported bound to several plasma proteins, and appear to exert their effect through influencing growth hormone synthesis (Lawrence and Fowler, 1997).

Anabolic Steroids

The steroid hormones such as progesterone, testosterone and estradiol act on gene expression rather than enzyme activity (Stryer, 1988), that is to say they stimulate synthesis of certain proteins rather than alter the activation of existing molecules. The effect of testosterone in farm species is widely known, through the effects of castration. Testosterone stimulates muscle protein synthesis, possibly mediated via hormone specific receptors which react with DNA (Buttery, 1983). Castration increases fattening, and treatment with exogenous testosterone tends to decrease fat deposition (Roche and Quirke, 1986). The anabolic effects of Oestradiol have been suggested to be mediated by increasing the levels of circulating endogenous hormones (Buttery and Sinnet-Smith, 1982). The corticosteriods have catabolic effects on protein metabolism in muscle (Buttery, 1983), and can be strongly lipogenic in adipose tissue (Lawrence and Fowler, 1997).

β-agonists

β- adrenergic agonists are powerful repartitioning agents, and have been found to increase muscle accretion whilst decreasing fat deposition (Moloney *et al.*, 1991; Dawson *et al.*, 1991; Sinclair *et al.*, 1991). Dawson *et al.* (1993) reported results which suggested that the rate of fat synthesis was reduced in animals treated with cimaterol, whilst others have reported evidence of an increase in the rate of lipolysis in treated animals (Beermann *et al.*, 1987; Mersman, 1987; Peterla *et al.*, 1987). Increases in muscle protein synthesis in response to treatment with β-agonists have been reported (Emery *et al.*, 1984; Claeys *et al.*, 1989) as have decreases in muscle protein breakdown (Borohov *et al.*, 1987; Dawson *et al.*, 1988).

Catecholamines

Catecholamines such as acetylcholine, epinephrine and dopamine have been shown to decrease acetyl Co-A carboxylase activity, thereby decreasing the rate of lipogenesis. They also stimulate lipolysis via activation of hormone sensitive lipase (Vernon, 1992), and this effect is increased *in vivo* by treatment with GH (Vernon *et al.*, 1991).

1.8.6. Manipulation of carcass composition.

There are a number of means, both nutritional and physiological that have important roles in controlling carcass composition in ruminants.

1.8.6.1. Feed restriction

Most attempts to use nutritional means to manipulate carcass composition have been via feed restriction at different growth stages, rather than through altering the pattern of nutrients available to the tissues. Ball *et al.* (1997) reviewed this area and concluded that response of animals to feed restriction depends on the severity and timing of restriction. The closer the animal is to maturity, the higher the fat deposition relative to lean (Searle *et al.*, 1972). Therefore restricting feed supply in the finishing stage of ruminant growth would seem to be a convenient tool in limiting fat deposition in the carcass. Indeed the most common effect of restricting energy supply seems to be a decrease in fat deposition, while protein deposition was unaffected (Bass *et al.*, 1990, Marias *et al.*, 1991, Murphy and Loech, 1994). However other workers have found either no effect of energy restriction on body composition (Kellaway, 1973; Drennan, 1979), or an increase in fat deposition with a decrease in lean (Butler-Hogg and Johnsson, 1986; Greef *et al.*, 1986b). In these cases the response to restriction may have been altered by the pattern of protein and energy yielding nutrients that were available to the animal.

The visceral organs play a major role in energy efficiency. Although they occupy only 0.1 of total body mass, they contribute 0.4-0.5 of total energy expenditure (Birkelo, 1995). Therefore any nutritional manipulation which affects visceral organ size will clearly have an effect on the overall energy metabolism of the animal. Animals exhibiting compensatory growth will replenish visceral organs as a priority over carcass gain (Ryan *et al.*, 1993). Ball *et al.* (1997) suggested that rather than comparing treatment means for visceral organ weight, a comparison of the variation in weights within treatments may be more appropriate as it has been observed that restricted fed animals exhibit a greater variation in visceral

organ weight, which may contribute to a greater variation in overall efficiency of energy utilisation in these groups and therefore a greater variation in carcass composition.

It has been indicated that restriction of feed down to about 0.80-0.85 of *ad lib* intake may improve the efficiency of utilisation of feed for growth (Sainz, 1995). The majority of the improvement may be accounted for by the change in the fat:lean proportion of gain, as the energy cost of fat deposition is 38.6MJ/kg and that of lean is 23.7MJ/kg (Webster, 1980). However the maintenance of lean tissue carries a higher energy cost than the maintenance of fat (Webster, 1980) so lean, restricted-fed animals may be expected to have a higher maintenance requirement. However it seems that restricted animals adapt to restriction by decreasing their maintenance requirement, possibly through a reduction in visceral organ mass (Ball *et al.*, 1997).

1.8.6.2. Pattern of nutrient supply

Few studies have looked at the effect of relative supply of energy and protein on N balance. Wilkinson and Greenhalgh (1991, 1995) investigated whether the pattern of forage and concentrate supply affected the growth of lambs. They found that feeding fixed amounts of roughage and concentrate simultaneously, progressively or separately resulted in no significant differences in final empty body weight.

In general it is agreed that at a fixed energy intake, N balance increases linearly up to a maximum for that level of energy intake (See Figure1.5). Where amino acid supply is limiting relative to energy supply, energy deposition will proceed in the absence of N deposition, i.e. the animal will lay down fat (Black and Griffiths, 1975; Ball *et al.*, 1997). In situations where the supply of amino acids is high relative to that of energy, at low energy intakes, protein deposition will be maintained at the expense of body fat, which could be a mechanism of manipulating body composition (Vipond *et al.*, 1989; Ball *et al.*, 1997). However this nutritional strategy has the drawback of also restricting overall growth. At

higher energy intakes, increasing the relative supply of amino acids can lead to an increase in fat deposition through the supply of glucogenic precursors from the amino acids (Oddy *et al.*, 1997).

Feeding a diet resulting in a low acetate:propionate ratio has been shown to cause increases in both fat and protein deposition when a source of DUP (for example fishmeal), was fed (Abdul-Razzaq and Bickerstaffe, 1989). A high molar proportion of propionate will promote a higher plasma insulin concentration, and insulin is known to promote storage of energy as fat (Vernon, 1992), and also to increase protein synthesis whilst having no effect on protein degradation, thereby increasing N retention (Reeds and Davis, 1992). This is supported by the work of van Houtert and Leng (1993) who reported that lambs given an intra-ruminal infusion of propionate and protected casein had higher growth rates than lambs given infusions of protected casein with either acetate alone or a mixture of acetate and propionate. However Beauchemin *et al.* (1995), by feeding diets differing in energy and protein supply, and protein degradability concluded that altering dietary regime had limited potential to affect carcass leanness on a high concentrate diet.

1.9. RUMEN SYNCHRONY.

1.9.1. The Concept of Rumen Synchrony.

Feed ingredients are digested at various rates in the rumen, resulting in N and energy becoming available to the rumen microbes at differing times. Figure 1.6. shows some theoretical rates of release of N and OM from different feed ingredients. Curves A, B and C could represent N release from soluble, quickly degradable and slowly degradable protein sources respectively. Curves X, Y and Z could represent release of energy from sugars, starch and fibre respectively. Rumen bacteria require energy and protein to be available simultaneously to stimulate rapid growth (Huber and Herrera-Saldana, 1994), so synchronisation of N and OM supply to the rumen has been proposed as a technique to increase bacterial growth rates and efficiency of nutrient utilisation. A diet containing an energy source releasing OM according to curve Y, and a protein source releasing N according to curve B would be synchronous. One in which OM release followed curve X and N release followed curve C would be highly asynchronous. For maximum efficiency of microbial protein production the N:OM ratio needs to be maintained at a constant level of 25gN/kg organic matter truly digested in the rumen (OMTDR) (Czerkawski 1986).

In order to achieve this, Sinclair *et al.* (1993) used *in situ* degradability coefficients to calculate the quantity of N and OM degraded in the rumen per hour, and compare this to the optimum ratio of N:OM. From this a synchrony index was calculated according to equation 1.1.

$$\frac{25-\sum_{1-24}\frac{\sqrt{(25\text{-hourly N/OM})^2}}{24}}{25} \qquad (1.1)$$

where 25 = 25 g N/kgOM truly digested in the rumen, which is assumed to be the optimum ratio for microbial protein synthesis (Czeralwski, 1986). A synchrony index of 1.0 represents perfect synchrony between energy and nitrogen, i.e. 25gN are released in the rumen for every kg OM in every hour of the day. A synchrony index of <1.0 indicate the degree of asynchrony.

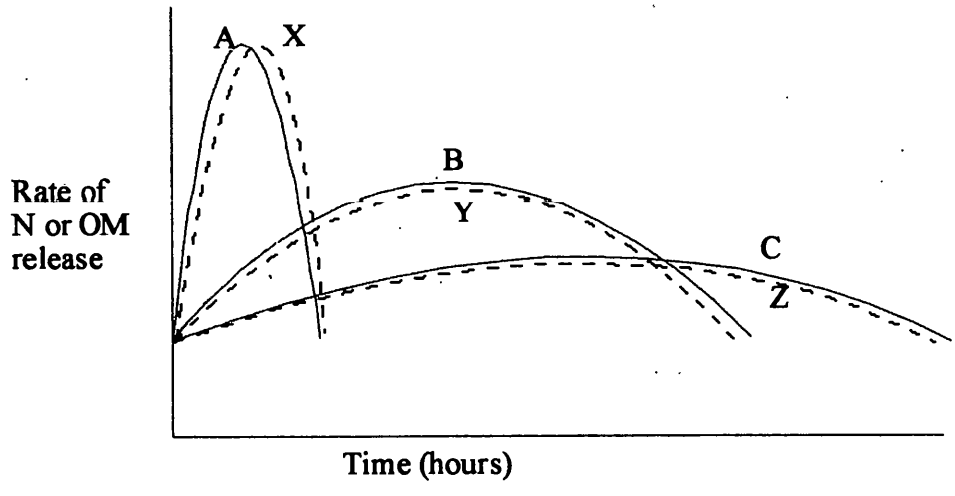


Figure 1.6. Theoretical rates of N (curves A, B, C) and OM (curves X, Y, Z) release in the rumen from highly soluble (A, X), intermediate (B, Y) and slowly degradable (C, Z)feed ingredients.

1.9.2. Effect of Synchrony on Rumen Metabolism.

Several studies have indicated that synchronising the supply of energy and N to the rumen on an hourly basis may increase the efficiency of microbial protein synthesis (Rooke and Armstrong, 1989; Sinclair *et al.*, 1993, 1995; Kim *et al.*, 1999).

1.9.2.1. In vitro *studies*

Henning *et al.* (1991) investigated the effect of synchronous and asynchronous supplies of energy and nitrogen on the growth of rumen bacteria *in vitro*. They found that the major factor influencing bacterial growth was the rate of energy supply, with the fastest rate of supply giving the highest efficiency, and that synchronising the supply of nitrogen had no significant effect. Newbold and Rust (1992) found that supplementation of equal amounts of energy and protein in synchronous or asynchronous patterns over 12 hours *in vitro* resulted in greater bacterial population size on the synchronous supply in hours 5 to 8 of the incubation, but that by 12 hours, when the total amounts of energy and protein supplied to each treatment were equal, no difference in population size could be detected. It was noted that bacterial populations recovered quickly from transient deficits of nitrogen, which goes some way towards supporting the theory that the pattern of supply of nitrogen is not a critical factor affecting microbial growth, assuming the total supply per unit time (eg. g/day) is adequate.

Infusion studies

Much work has been carried out to investigate whether the efficiency of utilisation of silage N can be improved by supplementation (Rooke *et al.*, 1985; Chamberlain *et al.*, 1985, Rooke and Armstrong, 1989). During ensilage the soluble carbohydrates in the forage are fermented by anaerobic bacteria to yield lactic acid and volatile fatty acids, and proteins are extensively degraded to amino acids and ammonia (Rooke *et al.*, 1987). When the silage is fed to the animal, the main carbohydrate sources available to the rumen microorganisms are slowly fermented structural carbohydrates, and the main N sources are highly soluble. Therefore silage could be described as being a highly asynchronous diet. Rooke *et al.* (1987) reported that supplementation with glucose syrup resulted in a decrease in rumen ammonia concentrations, and an increase in non-ammonia N flow to the duodenum. Both this (Rooke *et al.*, 1987) and a later study (Rooke and Armstrong, 1989) suggest that the improvement in microbial protein synthesis may have been due to a greater degree of synchronisation between N and energy supply to the rumen microorganisms.

Henning *et al.* (1993) repeated their earlier *in vitro* work (Henning *et al.*, 1991) *in vivo*, using either continuous infusion or pulse doses of energy and nitrogen to mimic slow and fast rate of release of nutrients. They reported that the degree of synchronisation did not affect microbial growth or efficiency, which was in agreement with their earlier *in vitro* study. However, in contradiction to the earlier work (Henning *et al.*, 1991), a slow release of energy, i.e. continuous infusion, produced a higher efficiency of microbial growth than a rapid release. Continuous infusion also resulted in a higher rumen pH and lower concentration of lactic acid. The results from these two studies (Henning *et al.*, 1991, 1993)

indicate that it is the energy supply which is most critical to microbial growth, and that *in vivo* a continuous supply of energy may be beneficial in order to maintain a steady rumen environment.

Feeding studies

Most studies into the effects of feeding synchronous diets on rumen metabolism have been carried out using dairy cows (Tables 1.3 and 1.4). Herrera-Saldana *et al.* (1990a) observed a greater flow of microbial protein to the duodenum in dairy cows fed a synchronised rapidly degradable diet, than on a synchronised slowly degradable diet, or unsynchronised diets. This study also concluded that starch degradability affected utilization of nutrients in the rumen more than protein degradability, with the rapidly degradable starch source (barley) producing a higher synthesis of microbial N than the more slowly degradable source (milo). Similarly Aldrich *et al.* (1993) reported that passage of bacterial nitrogen to the duodenum was greatest when high rumen available carbohydrate was combined with high rumen available protein (i.e. synchronous diet) and lowest when high rumen available carbohydrate was combined with low rumen available protein (ie. asynchronous diet). In contrast to these studies Casper *et al.* (1999) reported that there was no effect of energy source (corn *vs.* barley), protein source (soya bean meal *vs.* extruded soya bean meal) or interaction on rumen pH, VFA concentration or rumen bacterial synthesis in lactating cows. Kim *et al.* (1999) also reported no effect of synchrony on microbial protein synthesis in cattle fed grass silage.

Shabi *et al.* (1998) reported that increasing ruminal availability of OM caused a decrease in rumen ammonia and plasma urea N in dairy cows, and although no significant increase in microbial synthesis was seen, there was an increase in the abomasal flow of crude protein.

They concluded that for the maximum efficiency of ruminal nitrogen utilisation frequent feeding of a highly degradable OM source is the best strategy, and that the pattern or source of N supplementation is of lesser importance.

The effects of nutrient synchrony in the rumen have also been examined in sheep. Sinclair *et al.* (1993) fed synchronous and asynchronous diets to sheep, and found that the synchronous diet resulted in higher average rumen ammonia concentrations, and more constant rumen VFA proportions than the asynchronous diet. This corresponded with a higher efficiency of microbial protein synthesis. These improvements can be attributed to the fact that the synchronised diet resulted in an improved pattern of supply of nitrogen and energy to the rumen microbes, and a more stable rumen environment. However, the two diets used in this study were composed of different raw materials, the synchronous diet containing a much greater proportion of rapidly degradable starch in the form of barley, than the asynchronous diet. As has already been mentioned, rapidly degradable energy sources result in higher microbial N synthesis than slowly degradable sources, and it may be that the differences found between the two diets could be attributed to this fact rather than to the degree of synchrony. Sinclair *et al.* (1995) attempted to overcome this objection by formulating two diets similar in carbohydrate composition one of which was synchronous, and one asynchronous. It was found that the efficiency of microbial protein synthesis was significantly greater in the animals fed the synchronous diet. Similarly, when examining a range of forages fed to sheep, Verbič *et al.* (1999) reported a positive correlation between microbial N supply to the animal and degree of synchrony

The effects of synchronising nitrogen and energy supply to the rumen on microbial protein supply and microbial efficiency are summarised in Tables 1.3 and 1.4.

Table 1.3. The effect of synchronising energy and nitrogen supply to the rumen on microbial protein synthsis (g/d).

Species	Fast energy/ fast nitrogen	Fast energy/ slow nitrogen	Slow energy / fast nitrogen	Slow energy/ slow nitrogen	Reference
Cattle	262[a]	214[b]	234[b]	237[b]	Aldrich *et al.*, 1993
	275	295	282	312	Casper *et al.*, 1999
	310[a]	282[a]	294[a]	259[b]	McCarthy et al., 1989
	234	200	206	213	Shabi *et al.*, 1998
Sheep	7.8[a]	4.8[b]	6.2[b]	5.2[b]	Witt *et al.*, 1999b

Values within rows with different superscripts differ ($p<0.05$)

Table 1.4. The effect of synchronising energy and nitrogen supply to the rumen on the efficiency of microbial protein synthesis in cattle (gN/kg OM truly degraded in the rumen).

Fast energy/ fast nitrogen	Fast energy/ slow nitrogen	Slow energy / fast nitrogen	Slow energy/ slow nitrogen	Reference
17.9[a]	14.5[b]	15.2[a,b]	17.8[a]	Aldrich *et al.*, 1993
52.6	52.8	58.6	51.3	McCarthy *et al.*, 1989
25.8	25.3	24.8	30.9	Shabi *et al.*, 1998

Values within rows with different superscripts differ ($p<0.05$).

1.9.3. Effect of synchrony on intake.

Studies into the effects of synchrony on dry matter intake (DMI) in sheep have tended to use silage-based diets (Blackburn *et al.*, 1998; Witt *et al.*, 2000). Blackburn *et al.* (1998) reported that the pattern of N and OM release in the rumen appeared to have little effect on the pattern of DMI in growing lambs, and Witt *et al.* (2000) reported no differences in DMI of lactating ewes fed silage-based complete diets which were formulated to be either synchronous or asynchronous. In cattle, Aldrich *et al.* (1993) and Casper *et al.* (1999)

reported that energy source within the diet affected DMI, but that synchronising N and OM supply had no effect which supports the theory that the differences in DMI in growing lambs reported by Witt *et al.* (1999a) may have been due to energy source rather than synchrony. In contrast Herrera-Saldana *et al.* (1990) reported that lactating cows fed synchronous diets had lower DMI than those fed asynchronous diets, although this was not statistically significant.

1.9.4. Effect of synchrony on production characteristics.

Several studies have been carried out into the effect of dietary synchrony on lactation (Table 1.5). In general it seems that feeding a rapidly degradable energy source either decreases (Herrera-Saldana and Huber, 1989; Casper *et al.*, 1999), or has no effect on (Moorby *et al.*, 1996) milk fat production and increases milk protein production (Aldrich *et al.*, 1993; Moorby *et al.*, 1996; Shabi *et al.*, 1998). Synchronisation of N and energy supply has generally been reported to have little effect on milk production or composition (Kolver *et al.*, 1998; Casper *et al.*, 1999; Witt *et al.*, 2000). In contrast to these studies, Kim *et al.* (1999) reported that in dairy cows receiving intraruminal infusions of maltodextrin designed to be either synchronous or asynchronous with nutrient release from the basal diet, milk fat and protein yield were both lower on the synchronous infusion pattern. Similarly, Henderson *et al.* (1998) reported that milk CP yield was higher in cows fed an asynchronous silage based diet than in cows fed a synchronous diet.

Table 1.5. The effect of synchronising organic matter and nitrogen supply to the rumen on milk production

	Fast energy/ fast nitrogen	Fast energy/ slow nitrogen	Slow energy / fast nitrogen	Slow energy/ slow nitrogen	Reference
Milk yield (kg/d)	39.3	38.8	39.5	39.6	Aldrich *et al.*, 1993
	23.8^{a}	23.5^{a}	$25.1^{a,b}$	27.5^{b}	Casper *et al.*, 1999
	32.6^{a}	32.4^{a}	35.2^{b}	35.9^{b}	McCarthy *et al.*, 1989
	18.5	18.2	17.5	18.2	Shabi *et al.*, 1998
Milk protein %	3.08^{a}	3.05^{a}	3.04^{a}	2.98^{b}	Aldrich *et al.*, 1993
	3.32	3.24	3.24	3.32	Casper *et al.*, 1999
	3.17	3.14	3.15	3.21	McCarthy *et al.*, 1989
	3.5	3.5	3.4	3.4	Shabi *et al.*, 1998
Milk fat %	3.26	3.40	3.46	3.38	Aldrich *et al.*, 1993
	2.66	2.76	2.80	2.66	Casper *et al.*, 1999
	3.04	2.89	2.73	2.72	McCarthy *et al.*, 1989
	3.4	3.7	3.7	3.7	Shabi *et al.*, 1998

Values within rows with different superscripts differ ($p<0.05$)

The effects of synchrony of nutrient release in the rumen on growth has not been investigated so extensively. Matras *et al.* (1991) investigated the effects of feeding energy and protein sources varying in degradability on N utilisation in growing lambs. They reported that feeding synchronous diets, i.e. rapidly degraded sources of N and OM together or slowly degraded N and OM sources together, resulted in the most efficient N utilisation (g N retained/g N intake). Witt *et al.* (1999b) fed synchronous and asynchronous diets at a restricted level to growing lambs. It was found that a higher degree of dietary synchrony produced significantly improved growth rates and feed conversion ratios. Carcass characteristics were not affected by degree of synchrony, with the exception of

kidney fat, which was increased on the synchronous diet, indicating differences in efficiency of energy use. When the same diets were fed *ad lib* to growing lambs (Witt *et al.*, 1999a) lambs fed diets containing a rapidly degradable energy source grew significantly faster than those on a slowly degradable energy source, but there was no effect of energy and N synchronisation in the rumen. However the synchronous fed lambs did have a significantly higher feed conversion efficiency than lambs fed asynchronous diets.

Previous studies investigating the effects of synchronising energy and N supply to the rumen have compared diets differing in energy source (Matras *et al.*, 1991; Witt *et al.*, 1999a, b). As energy source within the diet is known to have a significant effect on production (Herrera –Saldana and Huber, 1989; Aldrich *et al.*, 1993; Casper *et al.*, 1999) it is possible that the effects on growth reported by Witt *et al.* (1999a, b) may have been due to energy source rather than synchrony. The present study aimed to investigate the effects of both energy source and degree of synchrony within energy source on growth and metabolism in lambs.

CHAPTER 2.

MATERIALS AND METHODS

2.1. ROUTINE ANALYSIS

2.1.1. Dry Matter

Dry matter analysis was carried out according to the method of MAFF (1986). Sub-samples of diets (5g) were bulked and oven dried to a constant weight at 80°C. Bulked faecal samples were sub-sampled and dried to a constant weight at 80°C. Carcass and non-carcass samples were homogenised (Tecator Homogeniser-1094. Foss UK ltd, Parkway House, Station Road, Didcot, Oxon, OX11 7NN) sub-sampled and freeze dried to constant weight (Edwards 4K Modulyo freeze drier. Edwards High Vacuum International, Manor Royal, Crawley, West Suissex, RH10 2LW). Prior to analysis freeze dried samples were placed in liquid nitrogen until frozen (approximately 15 seconds) then homogenised in a food processor (Toshiba TFP 1200). To ensure this method homogenised the sample adequately and produced consistent samples, organic matter (OM) determination was carried out on 10 samples from one carcass. The mean and standard deviation are presented in Table 2.1.

Table 2.1. Consistency of homogenised carcass and non-carcass samples.

Mean OM content of 10 homogenised carcass samples (g/kgDM)	905.24
Standard deviation	6.651

All subsequent analyses were carried out using dried material.

2.1.2. Organic Matter

Organic matter (OM) content of feed, residues, faecal and carcass samples was determined by the method of MAFF (1986). Approximately 2g of sample was weighed into a pre-weighed silica crucible and ashing at 550°C overnight in a muffle furnace (Gallenkamp Muffle furnace Size 3 GAFSE 620. Gallenkamp, Belton Road West, Loughbrough, Leics., LE11 0TR). Ashed samples were cooled to room temperature in a dessicator and re-weighed. The weight of the residue was recorded as the ash content, and OM content was calculated as;

$$OM\ (g/kgDM) = 1000 - Ash(g/kgDM) \qquad (2.1.)$$

2.1.3. Nitrogen

2.1.3.1. Total Nitrogen

Total nitrogen was determined using the Kjeldahl method according to MAFF (1986). Approximately 1g of feed, residue, faeces, carcass or non-carcass sample was weighed into a filter paper envelope (Whatman no.1. Whatman Ltd, St Leonards Road, 20/20 Maidstone, Kent, ME16 0LS) and placed in a digestion tube with 15ml of 98% (w/v) low nitrogen, sulphuric acid and copper sulphate as a catalyst. In the case of urine, 5 ml of bulked sample was accurately pipetted into the digestion tube with the sulphuric acid and copper sulphate. Tubes were then placed on a digestion block in a fume cupboard and digested at 450°C for 55 minutes. After cooling for 15 to 20 minutes 75ml of deionised water was added to each tube. The digest was then made alkaline by addition of of sodium hydroxide and distilled into a

solution of 4% (w/v) Boric acid, before titration with 0.1M hydrochloric acid (Kjeltec 1035 autoanalyser. Foss UK Ltd, Parkway House, Station Road, Didcot, Oxon, OX11 7NN). Calculation of the nitrogen content of the sample was as shown below;

1ml of 0.1 HCl = 0.0014g N

$$\text{Nitrogen (g/kg)} = \frac{1.4 \times \text{volume of HCl (ml)}}{\text{Sample weight (g)}} \qquad (2.2.)$$

A factor of 6.25 was used to convert N content to crude protein content.

2.1.3.2. Rumen Ammonia Nitrogen

Ammonia nitrogen content of the rumen fluid samples was determined by centrifuging the samples (4°C, 28600xg for 25 minutes; Beckman Avanti 30 centrifuge. Beckman Instruments (UK) Ltd, Oakley Court, Kingsmead Business Park, High Wycome, Buckinghamshire, HP11 1UJ) and accurately pipetting 10ml of supernatant into a digestion tube. Magnesium oxide suspension (6ml; 17g ignited magnesium oxide in 100ml distilled water) was added and the solution made alkaline by the addition of sodium hydroxide and distilled into a solution of 4% boric acid, before titration with 0.02M hydrochloric acid (Tectator 1030. Foss UK Ltd, Parkway House, Station Road, Didcot, Oxon, OX11 7NN). Nitrogen content of the sample was calculated as;

$$\text{N (mg/l)} = \frac{14.01 \times 0.02}{\text{ml sample}} \times \text{volume of HCL(ml)} \times 1000 \qquad (2.3.)$$

2.1.4. Neutral Detergent Fibre

Neutral detergent fibre (NDF) content of feed and faeces was determined by the method of Van Soest *et al.* (1993). Approximately 0.5g of ground sample was accurately weighed into a previously dried and weighed Fibretec crucibe (P1). The crucible was placed in the Fibretec apparatus (Tecator Fibretec 1020 Hot Extractor. . Foss UK Ltd, Parkway House, Station Road, Didcot, Oxon, OX11 7NN) and 25 ml cold NDF reagent (93g disodium ethylene tetra-acetate dihydrate (EDTA), 34g sodium borate, 150g sodium lauryl sulphate, 50ml 2-ethoxy ethanol mixed with 22.8g anhydrous disodium phosphate made up to 5 litres and pH adjusted to pH7) added with 0.5ml of octanol. The sample was then heated to boiling, and digested for 30 minutes before the addition of a further 25ml of cold NDF reagent with 2ml α-amylase solution (2g α-amylase E.C.3.2.1.1. from *Bacillus sbtilis* in 90ml water, filtered and 10 ml 2-ethoxy ethanol added, stored at 4°C). The sample was again heated to boiling and digested for a further 30 minutes before being filtered and washed 3 times with 20ml hot de-ionised water. Once filtration was complete, a further 25 ml of hot de-ionised water was added with a further 2 ml α-amylase and allowed to stand for 15 minutes before being filtered and washed 3 times with 20ml of hot de-ionised water and finally with 20 ml acetone. The crucibles were dried at 100°C overnight, allowed to cool in a dessicator and weighed. Crucibles were then placed in a muffle furnace (Gallenkamp Muffle furnace Size 3 GAFSE 620) and ashed at 550°C for 4 hours, allowed to cool and reweighed. The NDF content of the feed was calculated as:

$$\text{NDF (g)} = (\text{crucible + dry fibre weight}) - (\text{crucible + ash weight}) \quad (2.4)$$

$$\text{NDF (g/kg DM)} = \frac{\text{NDF weight (g)}}{\text{Sample weight (g DM)}} \times 1000 \quad (2.5).$$

2.1.5. Ether extract

Ether extract was determined according to the method of MAFF (1986). Approximately 1g of feed, carcass or non-carcass sample was accurately weighed into a cellulose extraction thimble (Whatman) which was then plugged with de-fatted cotton wool. The thimbles were placed in the extraction unit (Tecator Soxtec 1043. Foss UK Ltd, Parkway House, Station Road, Didcot, Oxon, OX11 7NN), and immersed in petroleum ether (25 ml for feed samples, 40ml for carcass samples) in pre-weighed extraction cups. The solvent was then brought to boiling point and the sample extracted for 30 minutes or 60 minutes for feed and carcass samples respectively. The thimbles containing the samples were then raised out of the solvent and rinsed for a further 30 or 60 minutes for feed and carcass samples respectively. The final traces of solvent were evaporated off, and the extraction cups removed from the apparatus, allowed to cool in a fume cupboard and re-weighed.

Ether extract (EE) content of the sample was calculated as:

$$\text{EE(g/kgDM)} = \frac{(\text{Extraction cup + fat weight (g)}) - (\text{Extraction cup weight (g)})}{(\text{Thimble + sample weight (g)}) - (\text{Thimble weight (g)})} \times 1000 \quad (2.6)$$

2.2. GROSS ENERGY DETERMINATION

Approximately 0.5 g of bulked, homogenised feed sample was accurately weighed into a

crucible and the gross energy content determined using an adiabatic bomb calorimeter (Parr Isoperibol Calorimiter 240; Parr instrument Company, Moline, Illinois, 61265, USA.) according to the methods of AOAC (1980). Samples were analysed in duplicate, using annular sucrose as the calibrant.

2.3. RUMEN VOLATILE FATTY ACID ANALYSIS

Volatile fatty acids (VFA) were analysed according to the method of Ryan (1980). Frozen acidified rumen fluid samples were thawed at room temperature and thoroughly mixed before transfer to 50ml centrifuge tubes and centrifugation at 28600g for 30 minutes at 4°C (Beckman Avanti 30 centrifuge). A sub-sample (4.5ml) of the supernatant was transferred into a 15ml soveril tube and 0.5ml of internal standard (25mM phenol) added. The sample was vortexed for 10 seconds and then filtered through a 0.2µm pore size syringe filter (Whatmans nitrocellulose membrane filter) into vials ready for gas chromatography.

Volatile fatty acids were analysed by gas liquid chromatography on a 30m x 0.25mm internal diameter (DB-FFAP) column (J. W. Scientific, 91 Blue Ravine Road, Folsom, CA 95630 4714, USA). The chromatagraph was a Perkin Elmer 8500 with an AS3800 autosampler with flame ionisation detection and helium as the carrier gas (split 5:1). A 2µl sample was injected at 300°C. The column temperature was 110°C and increased at 10°C per minute up to 200°C where it was held for 17 minutes.

Volatile fatty acids were quantified using an integrator and phenol as the internal standard. Each major peak was identified by comparison of retention times with those of known standards. Linearity of response was confirmed using external standards of known

concentrations of volatile fatty acids.

2.4. PURINE DERIVATIVES.

Urine was analysed for purine derivatives at the Rowett Research Institute according to the methods of Chen *et al.* (1990) for uric acid, xanthine and hypoxanthine and Chen *et al.* (1993) for allantoin. The daily output of purine derivatives in urine was calculated and used to estimate the amount of microbial nitrogen absorbed according to the method of Chen *et al.* (1992) as described below. The total daily PD excretion is calculated as the sum of the excretion of allantoin, uric acid, and xantine plus hypoxanthine expressed as mmol/d. The quantitative relationship between absorption of microbial purines (mmol/d) and excretion in the urine (mmol/d) in sheep is described by the following formula;

$$Y = 0.84X + (0.150\ W^{0.75}\ e^{-0.25X})$$

Where; Y = PD excretion in urine (mmol/d)

X = absorbed microbial purines (mmol/d)

0.84 = recovery of absorbed purines as PD in urine

$(0.150\ W^{0.75}\ e^{-0.25X})$ = net endogenous contribution of PD to total excretion after correction for the utilisation of microbial purines by the animal.

The calculation of X from Y based on the above equation is the performed by means of the Newton-Raphson iteration process as shown below;

$$X_{(n-1)} = X_n + \frac{f(X_n)}{f'(X_n)}$$

Where; $f(X) = 0.84X + 0.150\ W^{0.75}\ e^{-0.25X} - Y$

$f'(X) = 0.84 - 0.038\ W^{0.75}\ e^{-0.25X}$

The calculation of intestinal flow of microbial N (gN/d) is then calculates as;

$$\text{Microbial N (gN/d)} = \frac{X \text{ (mmol/d)} \times 70}{0.116 \times 0.83 \times 1000} = 0.727X$$

where; 0.83 = digestibility of microbial purines

70 = N content of purines (mg N/mmol)

0.116 = ratio of purine N : total N in mixed rumen microbes.

2.5. BLOOD ANALYSIS.

Blood samples were collected into 7ml evacuated tubes (Vacutainer) containing the anticoagulants lithium heparin for urea analysis, potassium oxalate for ß-hydroxybutyrate, glucose and insulin analysis or potassium EDTA for ammonia analysis. Samples were centrifuged at 3000rpm for 15 minutes (Beckman Avanti 30 centrifuge) and plasma transferred to micro-centrifuge tubes for storage at –20°C. Samples to be analysed for ammonia were kept on ice and were analysed within two hours of sampling. Analysis for ammonia, urea, glucose and β-hydroxybutyrate was carried out using a Bayer Technicon RA-1000 autoanalyser (Bayer plc, Strawberry Hill, Newbury, Berkshire, GR14 1LA) and the methods of Sigma (Sigma Diagnostics, PO Box 14508, St Louis, MO63178, USA). Plasma insulin analysis was carried out at S.A.C Aberdeen using a 125I-labelled insulin double-antibody radioimmunoassay based on the method of Starr *et al.* (1970).

2.6. USE OF ARTIFICIAL FIBRE BAGS TO DETERMINE DEGRADABILITY.

Synthetic fibre bags were made from polyester fabric with pore size 40µm according to the dimensions shown in Figure 2.1. Bags were stitched at approximately 10 stitches per cm with polyester thread, then turned inside out and re-stitched. The stitching was then sealed using

silicone sealant.

Feed samples were ground through a 2.0mm screen and then sieved through a 45μm screen to remove all fine particles. Approximately 5.0g of this material was weighed into each bag which was sealed with polypropylene string. Bags were inserted into the rumen 30 minutes after the morning feed and removed after 2, 4, 6, 8, 12, 24, 48, 72 or 96 hours. Six bags were incubated in the rumen of each sheep at any time. Bags were then washed through the cold rinse cycle of a domestic washing machine (Creda 800 Superspeed). In addition, synthetic bags containing samples of the feed which had not been incubated in the rumen were machine rinsed to estimate the rapidly soluble component. After washing, bags were dried in an oven at 60°C and re-weighed. Sufficient bags were incubated for each raw material at each time point to provide a pooled weight of residue of 7g for each sheep. Feeds and residues were analysed for dry matter, organic matter and nitrogen as described in section 2.1.3.1.. The data obtained was fitted to a first order model of Ørskov and McDonald (1979):

$$p=a+b(1-e^{-ct})$$

where p is the cumulative amount degraded at time t, a is the readily soluble fraction, b is the fraction potentially degraded in the rumen, c is the constant rate of degradation of b and t is time in hours.

In order to determine the presence of a lag phase, the data was fitted to a first order model containing a lag phase:

$$p = a \quad \text{up to time } t_0$$

$$p=a+b(1-e^{-ct}) \quad \text{from time } t_0 \text{ onwards,}$$

where a, b, and c are as described above and t_0 is the lag phase (h).

The effective extent of degradation (P) was calculated hourly using the equation;

$$P = a+((bc)/(c+k))(1-e^{-(c+k)t})$$

where k is the fractional outflow rate of solids from the rumen.

For components that contained a lag phase, degradation was calculated using:

$P = a$ up to lag

$P = a+((bc)/(c+k))(1-e^{-(c+k)(t-lag)})(e^{-klag})$ from lag time onwards

The quantity degraded per hour was calculated as the difference between the cumulative degradation in two successive hours.

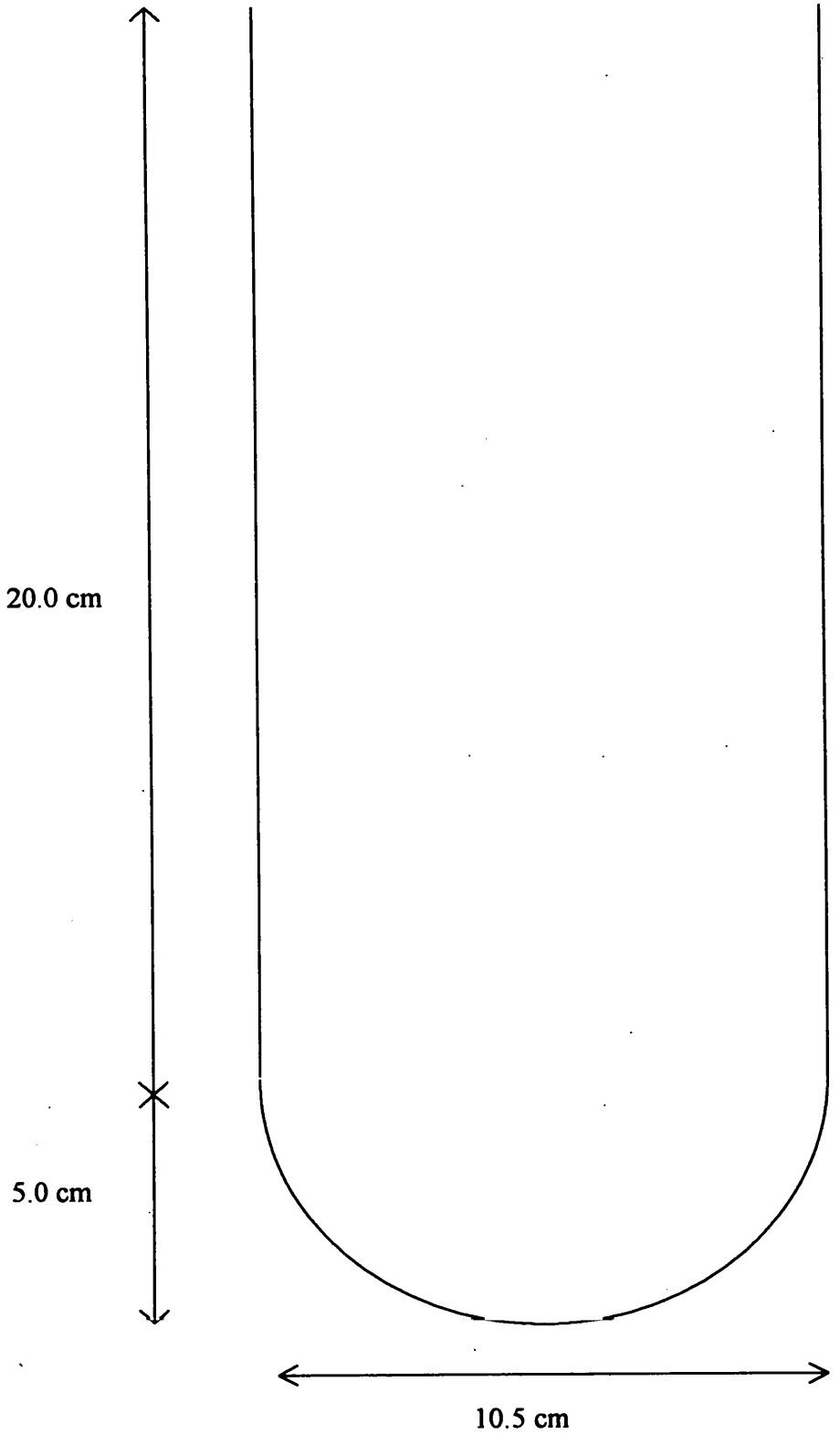


Figure 2.1. Dimensions of dacron bags.

CHAPTER 3.

DETERMINATION OF THE DEGRADABILITY OF FIVE FEEDSTUFFS USING ARTIFICIAL FIBRE BAGS INCUBATED IN THE RUMEN OF SHEEP

3.1. INTRODUCTION

In order to enable the alteration of the degree of dietary synchrony, accurate prediction of the hourly release of nitrogen and organic matter in the rumen from feed ingredients is required. Ørskov and McDonald (1979) used artificial fibre bags to estimate the degradability of protein in the rumen. Since then this method has been widely used to determine the degradability constants of various components of feedstuffs (Lycos and Varga, 1995; Susmel *et al.*, 1990; Herrera-Saldana *et al.*, 1990b). It may also be used to predict the pattern of nutrient supply to the rumen on an hourly basis (Sinclair *et al.* 1993).

The objectives of this experiment were:

1) to determine the degradability constants of nitrogen and organic matter for five feed ingredients using artificial fibre bags suspended in the rumen of cannulated wether sheep

2) to use this information to formulate diets which were synchronous or asynchronous with respect to their hourly nitrogen and organic matter release in the rumen.

3.2. MATERIALS AND METHODS

3.2.1. Animals and feeding procedures.

Four rumen cannulated wether sheep of approximately five years of age, with an average live weight of 79 kg (s.d. 4.5 kg) were housed in individual slatted floor pens under continuous lighting with free access to water and mineral lick blocks (Baby Rockies, Tithebarn Ltd, Southport, Merseyside. Composition; Sodium 38.0%, Magnesium 0.1%, Ash 98%, Iron 200mg/kg, Manganese 100mg/kg, Iodine 50mg/kg, Zinc 120mg/kg, Cobalt 100mg/kg, Selenium 20mg/kg). The five feeds used were: wheat straw (Cherwell Valley Silos, Banbury), barley (Shropshire Grain Ltd), unmolassed sugar beet pulp (Trident Feeds, Peterborough), rape seed meal (Cargill) and malt distillers dark grains (United Distillers). The basal diet was formulated using these ingredients and was predicted to supply 10.0MJ ME/kgDM and 158g CP/kg DM and to have an FME:ERDP ratio of 0.1. (Table 3.1.)

Table 3.1. Formulation of basal diet used in determination of degradability coefficients of five feed ingredients (g/kgDM).

Ingredient	
Wheat straw	347
Barley	173
Unmolassed sugar beet pulp	134
Rape seed meal	155
Malt distillers dark grains	156
Vitamins and minerals*	35

* Trouw Intensive Lamb, Trouw UK, Northwich, Cheshire. This provided on a total diet basis (g/kg) Ca, 1.6; P, 0.58; Na, 6.0: vitamins (mg/kg): retinol, 2.37; cholecalciferol, 0.033; α-tocopherol, 13: trace minerals (mg/kg): Fe, 26; Co, 1.3; Mn, 26; Zn, 33; I, 2; Se, 0.2.

The diet was fed in two equal portions at 09.00h and 16.30h, to a level of 1.1x maintenance according to AFRC (1992) recommendations. The basal diet was fed for 3 weeks before commencing incubations.

3.2.2. Preparation of test feeds.

All feedstuffs under investigation were prepared and incubated as described in Section 2.5. The data obtained were fitted to a first order model of Ørskov and McDonald (1979), and the degradability constants determined as described in Chapter 2.

3.2.3. Spreadsheet construction.

Using the degradability coefficients obtained, a spreadsheet was written (Quattro Pro. Version 6.01) based on the program reported by Sinclair *et al.* (1993). This allowed formulation of diets and calculation of the hourly release of nitrogen and organic matter in the rumen and was used to calculate a synchrony index (SI). Contained in the spreadsheet was a database of the five feed ingredients to be used, including the proximate composition and degradability constants determined in the degradability trial. The database also contained the degradation characteristics of urea, which was assumed to be 90% soluble, with the remaining 10% (b) degraded at a rate of 0.5/h (c) (Sinclair *et al.*, 1993).

The spreadsheet required the user to input the proportion of each feed ingredient in the diet on a dry matter basis (g/kgDM), the quantity of feed given in each hour of the day (kg DM), and

the fractional outflow rate of solids (k) from the rumen. The spreadsheet then calculated and displayed the composition of the diet, including ME, OM and CP content, and the supply of nutrients from the diet, including fermentable ME, effective rumen degradable protein (ERDP), digestible undegradable protein (DUP) and metabolisable protein (MP) supply according to the equations of AFRC (1992). The spreadsheet also calculated and displayed both numerically and graphically the N:OM ratio in the rumen on a hourly basis over 24 hours. This information was then used to calculate the synchrony index (SI). This was achieved by calculating the amount of N and OM released in the rumen from each feed ingredient for each hour of the day, from each feeding time. For each ingredient at each feeding time the program was run until 150 hours post feeding, and each hour allocated to the appropriate hour of the day. For example, for the second hour of the day, the total amount of N or OM released in the rumen from a single feed ingredient fed in the first hour of the day was calculated as the sum of N or OM released in hours 2, 26, 50, 72, 98, 122 and 146. The total amount of N or OM released in that hour was then calculated as the sum of the N or OM released from every feed ingredient at every feeding time. This was carried out for every hour of the day, and the results used to calculate the synchrony index using Equation 3.1. (Sinclair *et al.*, 1993)

$$\frac{25-\sum_{1-24}\frac{\sqrt{(25\text{-hourly N/OM})^2}}{24}}{25} \qquad \text{Equation 3.1}$$

where 25 = 25gN/kg organic matter truly digested in the rumen, which is assumed to be the optimal ratio (Czerwalski 1986). A synchrony index of 1.0 represents perfect synchrony between nitrogen and OM release throughout the day. Values less than 1.0 indicate the degree of asynchrony.

3.3. RESULTS

The proximate analysis of the feed ingredients used is presented in Table 3.2.

Table 3.2. Proximate analysis of the five feed ingredients (g/kgDM)

	OM	NDF	CP
Wheat straw	935.6	679.0	38.6
Barley	977.9	114.1	86.9
Sugar beet pulp	844.8	372.7	84.4
Malt dark distillers grains	951.1	324.0	259.4
Rape seed meal	926.4	253.0	348.3

The crude protein contents of the two main energy sources (barley and sugar beet pulp) were very similar, whilst the organic matter content of the barley was higher than that of the sugar beet pulp. Of the two main protein sources, the organic matter contents were similar, but the rape seed meal had a higher content of crude protein than the distillers grains.

The degradability coefficients (*a*, *b* and *c*) are presented in Table 3.3. No lag periods were observed for N or OM for any of the feed ingredients. Large differences between feeds were observed in terms of the dry matter, nitrogen and organic matter degradability. Of the two main energy sources, barley had the greatest proportion of rapidly soluble OM (a) and also the highest rate of degradation (c) of the potentially degradable OM fraction (b). Sugar beet pulp had a relatively small rapidly degradable OM fraction (a), but the largest potentially degradable fraction (b), although the rate of degradation (c) was relatively low. Of the major N sources,

distillers grains had the highest rapidly degradable OM fraction (a), but had a smaller potentially degradable fraction (b) and a rate of degradation (c) very similar to that of rape seed meal. However the sum of the two fractions (a + b) was similar for each protein source (0.767 and 0.791 for distillers grains and rape seed meal respectively). Wheat straw had the lowest total degradable fraction (a + b) of all the feed ingredients, and the lowest rate of OM degradation (c).

Of the two major energy sources, sugar beet pulp had no rapidly degradable N fraction (a), and had a slower rate of degradation (c) of the potentially degradable (b) fraction than barley. Of the two major protein sources, the rapidly degradable N fraction (a) of distillers grains was more than double that of rape seed meal. Rape seed meal also had a much slower rate of degradation (c) of the potentially degradable fraction (b) than distillers grains. Wheat straw was estimated to have the slowest rate of N degradation (c) of all the ingredients, and the lowest potentially degradable N fraction (b). The values presented for wheat straw are those of Sinclair *et al.* (1993) as in the present study problems of microbial contamination of the incubated samples were encountered.

For each component, an analysis of variance was performed to give an indication of how significantly the individual feed ingredients differed from each other in their degradability characteristics. The r^2 value is also presented for each component of each feed ingredient to give an indication of how close each data point lay to the fitted curve.

Table 3.3. Calculated DM, OM and N degradability coefficients of the five feed ingredients determined by in sacco *incubation in the rumen of sheep.*

	a	b	c	a + b	lag	r^2
Organic Matter						
Wheat straw	0.076	0.605	0.026	0.681	0	98.9
Barley	0.489	0.424	0.213	0.913	0	94.8
Sugar beet pulp	0.065	0.872	0.077	0.937	0	98.6
Distillers grains	0.419	0.348	0.096	0.767	0	96.7
Rapeseed meal	0.287	0.504	0.098	0.791	0	98.3
s.e.d.	0.0081	0.0096	0.0322			
Nitrogen						
Wheat straw	0.207	0.37*	0.010*	0.577	0*	67.2*
Barley	0.338	0.559	0.134	0.897	0	97.2
Sugar beet pulp	0.000	0.923	0.032	0.923	0	93.5
Distillers grains	0.574	0.330	0.170	0.904	0	95.4
Rapeseed meal	0.253	0.645	0.079	0.898	0	97.5
s.e.d.	0.0083	0.0105	0.0091			

a = rapidly soluble component, b = potentially degraded fraction, c = rate of degradation of b, and lag is in hours.

* Values taken from Sinclair *et al.* (1993).

3.4. DISCUSSION

The five feed ingredients used were chosen to provide a range of degradation patterns for both OM and N to allow formulation of synchronous and asynchronous diets. Proximate analysis of the feed ingredients showed them all to have lower CP contents than the values reported by AFRC (1993), which resulted in the basal diet having a lower CP content than predicted (134gCP/kgDM *vs.* 158gCP/kgDM). The reported effects of dietary N supply on N and DM digestibility vary. Vik-Mo and Lindberg (1985) reported higher N and DM disappearance in animals fed high protein diets, whilst DeFaria and Huber (1984) reported no effects of diet CP content on DM disappearance from forages.

Whilst the degradability coefficients calculated for these ingredients were similar to those previously determined using the same sheep kept in identical conditions, using different batches of the same ingredients (Witt *et al.* 1999a), there were some marked differences. For example Witt *et al.* (1999a) found lag periods for OM in both straw and rape seed meal, whilst no lag periods were observed in the current work. The lag period is assumed to be the amount of time for cellulolytic bacteria to attach to the fibrous component of the feed (Akin, 1988; Susmel *et al.*, 1990). Therefore it can be suggested that in the current experiment the rumen microbes were able to attach more rapidly to the cellulose contained in the rape and straw.

The degradability coefficients for the OM component of wheat straw determined in the current study agree closely with those reported by Witt *et al*, (1999a) and Sinclair *et al.* (1993). Both the current study and that of Witt *et al.* (1999a) used the b, c and lag values for the nitrogen fraction of straw determined by Sinclair *et al.* (1993) due to problems of microbial contamination of the samples. The a, b and c values for the nitrogen fraction of wheat straw

published by AFRC (1993) are much higher that those used by the above mentioned authors.

Herrera-Saldana *et al.* (1990) found that the rapidly degradable (a) fraction constituted around 0.4 of barley dry matter, which is close to the 0.48 determined in the current trial. However there was a difference in N degradability in barley, with around 0.6 shown to be rapidly degraded in the study by Herrera-Saldana *et al.* (1990), and 0.45 in the study carried out by Sinclair *et al.* (1993) compared to only 0.34 in the current study. However values as low as 0.25 have been reported (Tamminga *et al.*, 1990).

Both the N and OM fractions of unmolassed sugar beet pulp showed similar patterns of degradation, with little or none being rapidly degraded, and the potentially degradable fraction (b) having a very low rate of degradation (c). This is in agreement with the findings of Witt *et al.* (1999a) who reported a similar rate and extent of degradation of unmolassed sugar beet pulp. However AFRC (1993) published a value of 0.24 for the rapidly degradable N fraction of sugar beet pulp.

The values determined in the current study for the organic matter component of distillers grains agree closely with those reported by Sinclair *et al.* (1993) and are similar to those reported by Witt *et al.* (1999a) for both batches of distillers grains studied. Those for the N fraction were also similar to those determined by Sinclair *et al.* (1993) and Witt *et al.* (1999a) with a significant proportion of the N being rapidly degraded (57% - 74%), and the remaining nitrogen being degraded at a slow rate.

The degradability coefficients determined for the OM fraction of rape seed meal broadly agree with those reported by Sinclair *et al.* (1993). Witt *et al.* (1999a) also reported similar values

for the 'b' and 'c' fractions to those reported in the current experinent, but a lower 'a' fraction in both of the rape seed meals studied. The degradability coefficients for the nitrogen fraction differ between studies with the rapidly soluble fraction in the current study (0.25) being greater than that reported by Witt *et al.*(1999a) for either of the rape seed meals studied (0.12 and 0.09), and that of Sinclair *et al.* (1993) who reported a value 0.17. However the current results are similar to those of Tamminga *et al.* (1990) who reported an 'a' fraction for rape seed meal of 0.21. Additionally the value published by AFRC (1993) of 0.32, is greater than either that of Tamminga *et al.* (1990) or the present study. Both the 'b' and 'c' fractions of rapeseed meal reported in the current trial are similar other reported values (Witt *et al.*, 1999a; Sinclair *et al.*,1993). IIowever both Tamminga *et al.* (1990) and AFRC (1993) report higher "c" values of 0.14 and 0.16 respectively compared with the value of 0.079 reported in the current experiment.

Previous authors have emphasised the need for prediction of the rumen degradation characteristics of each batch of feed ingredients when they are to be used in experimental diets to enable accurate prediction of the supply of N and OM to the rumen. Table 3.4 shows ERDP, DUP and MP supply from the basal diet used in the current experiment calculated according to the equations of AFRC (1993), using degradability coefficients determined in 3 different studies (Table 3.4).

Despite some differences in the absolute values of the degradability coefficients, the differences in predicted supply of ERDP, DUP and MP are very small. Additionally, the overall predicted ratio of N:OM release in the rumen would be 29.9, 29.2 and 28.8 using the coefficients of AFRC (1995), Witt *et al.* (1999a) and the current experiment respectively. However, the

calculated synchrony index would be 0.82, 0.83 and 0.94 respectively indicating that the pattern of N and OM supply to the rumen differs, despite similar total supplies. This serves to highlight the importance of characterising every batch of feed ingredients in experiments designed to investigate the effects of pattern of nutrient supply.

Table 3.4. Calculated supply (g/kgDM) of effective rumen degradable protein (ERDP), digestible undegradable protein (DUP) and metabolisable protein (MP) using degradability coefficients from three different studies.

Reference	Component	Calculated supply
AFRC (1995)	ERDP	83.1
	DUP	30.4
	MP	89.8
Witt *et al.* (1999a)	ERDP	78.7
	DUP	31.0
	MP	90.4
Current experiment	ERDP	78.5
	DUP	32.0
	MP	91.4

The dacron bag technique is widely used to estimate rumen degradability of a great variety of feedstuffs, but has been criticised for a number of reasons. There may be variations between laboratories in areas such as pore size of bags, sample preparation and size, and post incubation washing and drying of the residue. All of these factors influence the results obtained, and have been thoroughly reviewed by several authors (Nocek, 1988; Michalet-Doreau and Ould-Bah,

1992; Huntington and Givens, 1995). There are also fundamental criticisms of the method itself, including that microbial contamination of residues may lead to errors in the estimation of protein degradability (Nocek, 1988; Michalet-Doreau and Ould-Bah 1992) and it seems that the problem of microbial contamination is greater in forages and fibrous feeds than in concentrates. If microbial contamination causes errors in the estimation of protein degradability, it could potentially also lead to errors in the estimation of dry matter degradability. However Varvikko and Lindberg (1985) concluded that errors in dry matter disappearance are so small as to be of no biological significance.

In the current experiment all samples were ground through a 2mm screen and sieved prior to incubation. Emanuele and Staples (1988) suggested that milling of feed samples through a 2mm screen results in a normal distribution of particle sizes within a sample. Decreasing particle size may however increase the physical losses of undigested small particles from the bags, and so lead to over-estimations of the immediately soluble fraction (a). In order to minimise this error, all milled samples were sieved using a sieve with a mesh size of 45μm until no further material was lost. This should have ensured that all remaining feed particles had a minimum diameter of 45μm and were unable to pass undigested out of the bags which had a mesh size of 40μm. The ratio of sample weight : surface area used in the current study was 9.5mg/cm^2 which is within the range of 4 – 17mg/cm^2 quoted by Huntington and Givens (1995) as acceptable.

Despite criticisms, the *in situ* incubation of feedstuffs has been suggested to be the best current method of estimating digestion in the rumen as it provides the closest comparison with *in vivo* results (Huntington and Givens, 1995). Many *in vitro* techniques have been evaluated, and the continuous culture using rumen fluid does have the potential to offer significant savings of both time and money in evaluation of feedstuffs, although it has been proposed that *in vitro* methods may be useful for the ranking of feeds, rather than determining absolute values (Nocek, 1988). *In vivo* determinations have generally been the standard to which other techniques of calculating the extent of degradability are compared. However this method also has its disadvantages, not least that abomasally or duodenally cannulated animals are required. Contamination of digesta with endogenous protein, and variations in digesta flow can both contribute to the variability of results (Nocek, 1988).

Whilst the *in situ* technique does have a number of limitations, the method adopted in the subsequent metabolism and production experiments, that of supplying the same quantity of ingredients in different patterns within the day, minimises any possible problems arising from inaccuracies inherent in the prediction of the exact degradability coefficients.

CHAPTER 4.

THE EFFECT OF SEQUENCE OF CONCENTRATE ALLOCATION ON METABOLISM AND PERFORMANCE OF GROWING LAMBS FED AT A RESTRICTED LEVEL.

4.1. Introduction.

Feeding lambs diets which are formulated to be synchronous in terms of N and OM supply to the rumen has been shown to result in improvements in the efficiency of microbial protein production (Sinclair *et al.*, 1993, 1995). It has also been reported that in growing lambs synchronous diets resulted in increased growth rate (Witt *et al.* 1999a) and feed conversion efficiency (Witt *et al.* 1999b) and an alteration in energy metabolism. Other studies have indicated that the source and pattern of energy supply may be of greater importance to microbial efficiency than the degree of synchronisation between energy and nitrogen supply (Henning *et al.*, 1991; Henning *et al.*, 1993; Shabi *et al.*, 1998). However the diets used in a number of previous studies (e.g. Witt *et al.*, 1999a, b; Shabi *et al.*, 1998) were different in terms of the energy and protein sources used, and it is possible that the differences in performance observed were due to some aspect of diet composition that was not characterised rather than to the degree of dietary synchrony.

This study aimed to use diets that were identical in terms of their daily supply of each feed ingredient, but which differed in their hourly release of energy and N in the rumen. This was achieved by using the degradability constants for the raw materials presented in Chapter 3 and altering the sequence of allocation of the different components of the diet within the day in

order to alter the predicted pattern of N and OM release in the rumen. In order to investigate whether responses to synchrony were influenced by the energy source within the diet, two diets were used, one based on a rapidly degradable energy source (barley) and one on a more slowly degradable energy source (unmolassed sugar beet pulp).

4.2. Materials and Methods

4.2.1. Experimental Animals

Fifty-six entire male Suffolk cross lambs (Suffolk x (Friesland xLleyn) or (Suffolk x (Charrolais x Lleyn)), with an initial live weight of 25 kg (s.d. 4.2 kg) were used. Lambs were blocked according to breed and live weight, and then randomly allocated to one of six dietary treatments or to an initial slaughter group. Lambs were housed in individual slatted floor pens under continuous lighting with free access to water. The lambs were penned 12 days before the start of the trial, allowing 7 days adaptation to the pen while remaining on creep feed (Lambmaster, Wynnstay Farmers. 16% crude protein, 4% oil, 8% fibre, 6% ash) and five days for adaptation to the experimental diets. During the initial 7 day adaptation period lambs received creep feed *ad lib*. After 7 days the quantity of creep feed offered was reduced to experimental levels, and a 50:50 mixture of the two synchronous diets was introduced at a rate of 20% of total DM per day over a five day period. On day 0 of the trial all animals were changed onto the experimental diets.

4.2.2. Diets and feeding procedure

Using the same batches of ingredients, the degradability constants determined and the computer program described in Chapter 3, two diets were formulated, one based on barley (B), and one on unmolassed sugar beet pulp (S), to have a similar predicted metabolisable energy (ME), crude protein (CP) and metabolisable protein (MP) content (Table 4.1). Within each diet, the pattern of supply of different components of the diet was altered to give three different treatments, one synchronous (S) one intermediate (I) and one asynchronous (A) (Table 4.2). Diets were fed at 09.00h and 16.00h at a restricted level sufficient for 175 g/d live weight gain

according to AFRC (1995) recommendations. Lambs on the synchronous treatments received equal quantities of each raw material in each feed. Those on the intermediate treatment received the major slowly degradable protein source (rape seed meal) in the morning feed, and the majority of the rapidly degradable protein sources (distillers and urea) in the evening feed, whilst those on the asynchronous treatment received all of the major protein sources in the morning feed. Predicted ratios of N:OM release are illustrated in Figure 4.1. The allocation of straw was altered to ensure that all meals contained the same quantity of dry matter to minimise any potential fluctuations in rumen outflow rate. Within the barley-based diets the allocation of barley was equal in all meals to minimise any potential fluctuations in rumen pH. Therefore for each energy source, three treatments were produced which were identical in terms of quantity of each raw material supplied on a daily basis, but differed in the degree of synchrony

Table 4.1. Diet formulation and predicted chemical composition of two basal diets based on either barley or sugar beet pulp (SBP).

Ingredient (g/kgDM)	Barley diet	SBP diet
Unmolassed sugar beet pulp	0	412
Wheat straw	320	300
Barley	420	0
Distillers grains	164	60
Rape seed meal	60	199
Urea	12	5
Vitamins and minerals*	24	24
Total	1000	1000
Organic matter	934	875
Crude protein	146	145
Metabolisable energy (MJ/kgDM)	10.4	10.1
Fermentable ME (MJ/kgDM)	9.4	10
Effective rumen degradable protein	70	67
Digestible undegradable protein supply	31	39
Metabolisable protein supply	91	102

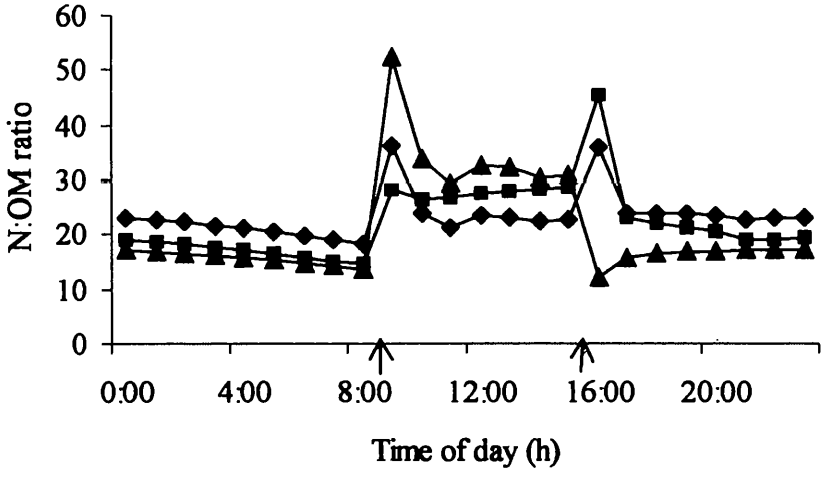
* Trouw Intensive Lamb, Trouw UK, Northwich, Cheshire. This provided on a total diet basis (g/kg) Ca, 1.6; P, 0.58; Na, 6.0: vitamins (mg/kg): retinol, 2.37; cholecalciferol, 0.033; α-tocopherol, 13: trace minerals (mg/kg): Fe, 26; Co, 1.3; Mn, 26; Zn, 33; I, 2; Se, 0.2.

All feed was consumed within one hour of feeding. The quantity of feed offered was recalculated weekly according to the live weight of the animal recorded on the previous day.

Table 4.2. Pattern of allocation within the day of raw materials from diets based on either barley (B) or sugar beet pulp (S) to give treatments which were synchronous (S), intermediate (I) or asynchronous (A) for their hourly release of nitrogen and organic matter in the rumen. (g/kgDM)

	BS	BI		BA	
	am & pm	am	pm	am	pm
Wheat straw	320	180	460	84	556
Barley	420	420	420	420	420
Distillers grains	164	256	72	328	0
Rape seed meal	60	120	0	120	0
Urea	12	0	24	24	0
Vits & Mins	24	24	24	24	24
Synchrony Index	0.864	0.760		0.624	
	SS	SI		SA	
	am & pm	am	pm	am	pm
Wheat straw	300	336	264	157	443
Sugar beet pulp	412	300	524	291	533
Distillers grains	60	0	120	120	0
Rape seed meal	199	340	58	398	0
Urea	5	0	10	10	0
Vits & Mins	24	24	24	24	24
Synchrony Index	0.885	0.760		0.610	

a)



b)

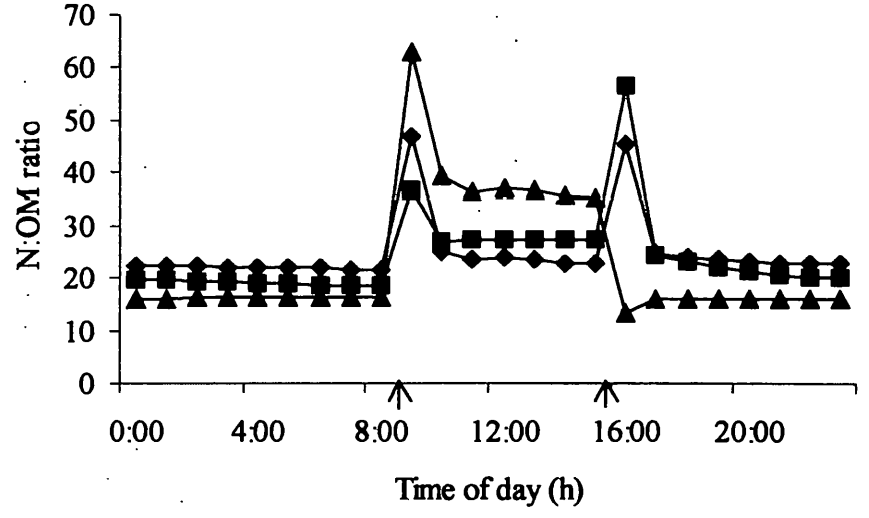


Figure 4.1. Predicted hourly ratios of N:OM release in the rumen of lambs fed diets based on either a) barley or b) sugar beet pulp and differing in sequence of allocation of individual ingredients to be synchronous (♦), intermediate (■) or asynchronous (▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate times of feeding.

4.2.3. Data collection.

4.2.3.1. Feed

Samples of each diet were taken fortnightly throughout the trial and bulked prior to analysis.

4.2.3.2. Live weight and carcass characteristics.

Lambs were weighed weekly at 14.00h and on reaching 40kg were sheared prior to slaughter. Daily live weight gain was calculated using regression. At slaughter, lambs were stunned, bled, skinned and eviscerated, and hot carcass weights were obtained within two hours. All carcass components were collected and weighed individually, and gut fill was determined by weighing the alimentary canal before and after emptying. Twenty four hours after slaughter the cold carcass weight was taken and kidneys and kidney fat were removed and weighed. Carcasses were then frozen at -20°C. The initial slaughter group were slaughtered on day 0 of the trial and processed as described.

4.2.3.3. Blood sampling

At 35 ± 2.8 kg live weight and having been on the experimental diet for a minimum of 6 weeks, six sheep from each treatment were blood sampled at 8.00, 10.30, 12.30, 15.00, 17.30, 19.30 and 22.00h. Blood samples were taken from the jugular vein by venepuncture into vacutainers containing the anticoagulants lithium heparin, potassium oxalate or potassium EDTA (section 2.4.). Samples were immediately centrifuged at 3000 rpm for 15 minutes and the stored at -20°C. Samples to be analysed for ammonia were immediately placed in an ice bath and analysed within 2 hours of sampling.

4.2.3.4. Rumen sampling

On reaching 38 ± 2.2 kg live weight rumen samples were taken from four sheep on each treatment. A total of six samples were taken over a two day period, via a stomach tube. Samples were taken at 8.00, 10.30, 12.30, 15.00, 17.30 and 19.30h. The first 20mls were discarded as being unrepresentative due to possible contamination with saliva, and the next 100mls collected. Samples were strained through a double layer of muslin, and the pH taken using a portable probe (Russel RL150). The samples were then acidified using concentrated HCl to a pH <3.0 and frozen at –20°C prior to subsequent analysis.

4.2.4. Chemical Analysis.

4.2.4.1. Feed

Feed samples underwent a full analysis according to the methods described in Chapter 2.

4.2.4.2. Carcass and non carcass components

The frozen half carcasses and non carcass components were ground separately, once through a 13mm screen and twice through a 4mm screen in a whole carcass grinder (Wolfking; Model c160-uni). The ground samples were mixed thoroughly and sub-samples taken (approx. 1kg). Samples were analysed for DM, OM, CP and EE as described in Chapter 2. Gross energy (GE) content of the whole body was determined by calculation, assuming that CP and EE had energy values of 23.6 and 39.9MJ/kg respectively (ARC, 1980). The CP, EE and GE content of each experimental animal on day 0 of the trial was predicted from live weight using regression equations based on the results obtained from the initial slaughter group.

4.2.4.3. Blood and rumen samples

Plasma samples were analysed for β-hydroxybutyrate, glucose, insulin, urea and ammonia as described in section 2.4.

Rumen fluid was analysed for ammonia and VFAs as described in sections 2.1. and 2.2.

4.2.5. Statistical analysis.

Results were analysed by analysis of variance using a two-way, randomised block design with

energy source and synchrony as the main effects. Each energy source was also analysed separately using a one-way randomised block design ANOVA. The blood and rumen data were also analysed using repeated measures ANOVA with energy source (barley *vs.* sugar beet pulp) and degree of synchrony (S, I, A) together with interactions forming the "between animal" stratum and sample time and associated interactions with energy source and synchrony forming the "within animal by time" stratum. All statistical analysis was performed using Genstat for Windows (Version 5, Release 3.22) and a significant difference was declared at $p<0.05$. The skeleton ANOVAs are presented below.

Table 4.3. Two way ANOVA used for statistical analysis of all growth, intake and carcass measurements.

Source	d.f.
Blocks	7
Energy source	1
Synchrony	2
Interaction	2
Residual	35
Total	47

Table 4.4. One way ANOVA used for statistical analysis of all growth, intake and carcass measurements.

Source	d.f.
Blocks	7
Synchrony	2
Residual	14
Total	23

Table 4.5. Two way ANOVA used for statistical analysis of all blood metabolites.

Source	d.f.
Blocks	5
Energy source	1
Synchrony	2
Interaction	2
Residual	25
Total	35

Table 4.6. One way ANOVA used for statistical analysis of all blood metabolites.

Source	d.f.
Blocks	5
Synchrony	2
Residual	10
Total	17

Table 4.7. Two way ANOVA used for statistical analysis of all rumen metabolites.

Source	d.f.
Blocks	3
Energy source	1
Synchrony	2
Interaction	2
Residual	15
Total	23

Table 4.8. One way ANOVA used for statistical analysis of all rumen metabolites.

Source	d.f.
Blocks	3
Synchrony	2
Residual	6
Total	11

Table 4.9. Repeated measures ANOVA used for statistical analysis of all blood metabolites.

Source	d.f.
Block	5
Energy	1
Synch	2
Energy . Synchrony	2
Residual	25
Time	6
Time. Energy	6
Time.Synchrony	12
Time . Energy . Synchrony	12
Residual	178
Total	251

Table 4.10. Repeated measures ANOVA used for statistical analysis of all rumen metabolites.

Source	d.f.
Block	3
Energy	1
Synchrony	2
Energy . Synchrony	2
Residual	15
Time	5
Time. Energy	5
Time.Synchrony	10
Time . Energy . Synchrony	10
Residual	90
Total	143

4.3. Results.

4.3.1. Feed analysis and nutrient intakes.

The proximate analysis for each of the experimental diets is shown in Table 4.3.

Table 4.11. Proximate analysis of diets (g/kg DM) based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (S) for their hourly release of nitrogen and organic matter in the rumen.

	BS	BI		BA	
	am & pm	am	pm	am	pm
Dry Matter (g/kg)	883.8	874.7	886.6	872.6	886.3
Organic matter	918.7	907.6	921.3	918.0	915.4
Crude protein	150.5	138.3	163.1	244.3	59.3
Ether extract	16.1	14.2	18.1	24.8	6.7
NDF	409.2	375.3	446.0	346.2	555.3
GE (MJ/kgDM)	18.0	16.7	18.4	18.8	17.3
	SS	SI		SA	
	am &pm	am	pm	am	pm
Dry Matter (g/kg)	886.3	891.1	883.7	881.4	893.7
Organic matter	883.0	884.0	888.8	892.6	884.4
Crude protein	152.6	153.0	147.7	232.7	68.6
Ether extract	14.9	14.1	15.1	21.2	9.3
NDF	499.6	540.8	542.8	427.2	619.4
GE (MJ/kgDM)	17.2	17.7	17.2	17.8	16.6

The synchronous diets (BS and SS) had OM and CP contents similar to those predicted. Supplying the slowly degradable protein in the morning feed and the rapidly degradable protein in the evening feed (treatments BI and SI) resulted in both the morning and evening feeds containing similar amounts (g / kg DM) of crude protein to the synchronous diets, whilst supplying all the major protein components of the diet in the morning feed (treatments BA and SA) resulted in 244g/kg DM and 233g /kg DM crude protein being supplied in the morning feed, and 59g/kg DM and 69g/kg DM CP being supplied in the evening feed for diets BA and SA respectively.

The NDF content of the barley based diets were lower than those of the sugar beet based diets (423.5g/kgDM *vs.* 540g/kgDM) due to the highly fibrous nature of sugar beet pulp compared to barley. Within the intermediate treatments, lambs on treatment BI received a greater quantity of NDF in the evening feed than in the morning feed (446g/kgDM vs. 375g/kgDM) whilst those lambs on diet SI received similar quantities of NDF in each meal. Both asynchronous treatments (BA and SA) supplied a greater quantity of NDF in the evening feed than in the morning feed. The two diets had similar EE contents with mean values of 16g/kgDM and 15g/kgDM for the barley and sugar beet based diets respectively. Both intermediate treatments supplied similar quantities of EE in each meal, whilst both asynchronous treatments (BA and SA) supplied a greater quantity of EE in the morning feed.

Table 4.12 Total nutrient intakes over the experimental period (kg) of diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (S) for their hourly release of nitrogen and organic matter in the rumen.

								Significance		
	BS	BI	BA	SS	SI	SA	s.e.d.	En	Syn	Int
DM	96.6	93.3	93.5	88.4	90.1	91.3	4.91	NS	NS	NS
OM	88.7	85.3	85.7	78.1	79.9	81.1	4.42	*	NS	NS
CP	14.5	14.1	14.2	13.5	13.6	13.9	0.74	NS	NS	NS
EE	1.56	1.51	1.47	1.31	1.32	1.40	0.076	***	NS	NS
NDF	39.5	38.3	42.1	44.2	48.8	47.6	2.39	***	NS	NS
GE	1745	1634	1691	1514	1572	1539	85.6	*	NS	NS

En = Effect due to energy source, Syn = Effect due to synchrony of N and OM supply to the rumen, Int = Interaction effect. * = $p<0.05$, ** = $p<0.005$, *** = $p<0.001$, NS = $p>0.05$.

Total nutrient intakes over the course of the trial are presented in Table 4.12. Intakes of organic matter, ether extract and gross energy were significantly lower in animals fed the sugar beet based diet than in those fed the barley based diet, whilst intakes of NDF were significantly

higher on sugar beet based diet than on the barley based diet. Within energy source, there were no significant differences between treatments in terms of nutrient intake over the duration of the trial.

4.3.2. Growth rate and feed conversion efficiency.

There were no significant differences between treatments in initial live weight, final slaughter weight, daily live weight gain (DLWG) or feed conversion efficiency (FCE)(Table 4.13.). Lambs on the barley based diets took approximately 7 days longer to reach slaughter weight than lambs on the sugar beet based diet ($p<0.05$).

Table 4.13. Daily live weight gain (DLWG) and feed conversion efficiency (FCE) of lambs fed diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (S) for their hourly release of nitrogen and organic matter in the rumen.

								Significance		
	BS	BI	BA	SS	SI	SA	s.e.d.	En	Syn	Int
Initial LW (kg)	24.6	24.4	25.1	25.8	25.3	24.4	0.73	NS	NS	NS
Slaughter wt (kg)	42.2	41.0	41.7	40.9	42.1	40.7	0.93	NS	NS	NS
Days on trial	93.4	91.6	90.7	83.7	85.5	85.5	5.01	*	NS	NS
DLWG (g/d)	182.4	185.1	188.0	182.5	193.5	184.4	11.8	NS	NS	NS
Intake (gDM/d)	1045	1047	1041	1084	1060	1106	29.7	NS	NS	NS
FCE (kg LWG /kg DMI)	0.18	0.18	0.18	0.17	0.19	0.17	0.012	NS	NS	NS

En = Effect due to energy source, Syn = Effect due to synchrony of N and OM supply to the rumen, Int = Interaction effect. * = $p<0.05$, ** = $p<0.005$, *** = $p<0.001$, NS = $p>0.05$.

Within the barley based diets, lambs fed the synchronous diet tended to have a higher cold carcass weight ($p<0.1$) than those fed either the asynchronous or intermediate diets (Table 4.14). Lambs on the barley based diet deposited significantly more kidney knob and channel fat than lambs on the sugar beet diet (mean values of 0.21 kg and 0.15kg respectively, $p<0.005$), whilst lambs on the synchronous treatments deposited significantly more than lambs on the

asynchronous treatments (mean values of 0.21kg and 0.15kg respectively, $p<0.05$). Lambs on the sugar beet based diets had a significantly greater weight of wool than lambs on the barley diets ($p<0.05$) whilst lambs on the barley based diet had significantly lower gut fill than lambs on the sugar beet diet ($p<0.05$). When the two energy sources were analysed independently, lambs on the synchronous barley diet (BS) had significantly heavier genitals ($p<0.05$) and significantly lower gut fill ($p<0.01$) than lambs on the asynchronous barley treatment (BA).

Table 4.14. Weights of carcass and non-carcass components (NC) (kg) of lambs fed diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (S) for their hourly release of nitrogen and organic matter in the rumen.

								Significance		
	BS	BI	BA	SS	SI	SA	s.e.d.	En	Syn	Int
Cold carcass	18.88	18.38	18.44	18.13	18.32	18.54	0.427	NS	NS	NS
Kill Out %	45.64	44.37	44.54	43.64	44.24	44.75	0.982	NS	NS	NS
Liver	0.675	0.687	0.681	0.728	0.729	0.676	0.0361	NS	NS	NS
Kidney	0.108	0.107	0.114	0.118	0.109	0.113	0.0051	NS	NS	NS
Kidney fat	0.257	0.203	0.169	0.171	0.158	0.134	0.0283	**	*	NS
Heart	0.174	0.169	0.172	0.179	0.172	0.168	0.0098	NS	NS	NS
Wool	1.179	1.115	1.119	1.253	1.259	1.307	0.974	*	NS	NS
Genitals	0.460	0.363	0.369	0.375	0.379	0.410	0.0458	NS	NS	NS
Empty gut	3.319	3.233	3.203	3.205	3.058	3.081	0.1675	NS	NS	NS
Gut fill	5.031	5.134	5.540	5.656	5.530	5.946	0.3027	*	NS	NS
Total NC	13.53	13.45	13.37	13.38	13.53	12.99	0.320	NS	NS	NS

En = Effect due to energy source, Syn = Effect due to synchrony of N and OM supply to the rumen, Int = Interaction effect. * = $p<0.05$, ** = $p<0.005$, *** = $p<0.001$, NS = $p>0.05$.

There were no significant differences in the water content of the carcass, although carcasses from lambs on the barley based diets had a significantly greater total dry weight compared to those lambs on the sugar beet based diet ($p<0.05$) with mean values of 7.47kgDM and 7.15kg DM respectively (Table 4.15). Within the barley diets, lambs on the synchronous treatment had significantly higher carcass dry weights than lambs on either the asynchronous or intermediate

treatments ($p<0.05$). There were no significant differences between energy source or degree of synchrony in either the ash content or the total quantity of OM deposited in the carcass. Carcasses from lambs fed the barley based diet had a significantly lower crude protein content ($p<0.05$), although this did not translate into a lower total mass of crude protein in the carcass. They also had a significantly higher ether extract content than lambs on the sugar beet diets, both in terms of g/kg DM ($p<0.05$) and total mass in the carcass ($p<0.01$). Carcasses from lambs on the asynchronous treatments tended to have a lower EE concentration than lambs on either the synchronous or intermediate treatments ($p= 0.069$).

Table 4.15. Carcass analysis of lambs fed diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (S) for their hourly release of nitrogen and organic matter in the rumen.

								Significance		
	BS	BI	BA	SS	SI	SA	s.e.d.	En	Syn	Int
Water (g/kg)	588.6	599.5	606.3	610.2	601.9	613.7	10.56	NS	NS	NS
Ash (g/kg)	48.9	45.5	50.0	44.9	45.4	49.8	3.91	NS	NS	NS
CP (g/kg)	173.2	173.7	178.0	178.6	177.4	181.8	2.93	*	NS	NS
EE (g/kg)	186.1	181.0	162.7	160.2	172.8	151.8	12.22	*	NS	NS
Total DM (kg)	7.79	7.35	7.27	7.06	7.31	7.08	0.245	*	NS	NS
Total OM (kg)	6.97	6.45	6.38	6.17	6.58	6.08	0.277	NS	NS	NS
Total CP (kg)	3.27	3.20	3.29	3.23	3.25	3.34	0.088	NS	NS	NS
Total EE (kg)	3.57	3.29	3.01	2.53	3.22	2.27	0.372	**	NS	NS

En = Effect due to energy source, Syn = Effect due to synchrony of N and OM supply to the rumen, Int = Interaction effect. * = $p<0.05$, ** = $p<0.005$, *** = $p<0.001$, NS = $p>0.05$.

Although there were no significant differences in water or ash content of the non carcass components, non carcass components from lambs fed the barley based diet had a significantly higher total non-carcass dry matter weight (4.45 *vs.* 4.23kg DM, $p<0.05$) and organic matter weight (4.15 *vs.* 3.90kg) than lambs fed the sugar beet based diets (Table 4.16.). Non-carcass components from lambs on the asynchronous treatments had a significantly lower total weight of dry matter than lambs on the intermediate or synchronous treatment (4.14 *vs.* 4.43 and 4.45kg DM for the asynchronous, intermediate and synchronous treatments respectively, $p<0.05$). Lambs on the synchronous sugar beet diet had significantly lower water content than lambs on the asynchronous sugar beet diet ($p<0.05$).

Non-carcass components from lambs on the barley based diet contained significantly less CP than those from lambs on the sugar beet based diet (158g/kg *vs.* 168g/kg for the barley and sugar beet based diets respectively, $p<0.01$), however when translated into total weight of non-carcass CP the difference was not significant. Non carcass components from lambs fed the barley based diet and a significantly higher fat concentration (132g/kg *vs.* 109 g/kg for barley and sugar beet based diets respectively, $p<0.001$) and a greater total weight of fat deposited in the non carcass components (1.78 *vs.* 1.46 kg for barley and sugar beet based diets respectively, $p<0.001$) than lambs on the sugar beet based diet. Non-carcass components from lambs on the asynchronous treatments had a significantly lower total EE content compared with lambs on the intermediate or synchronous treatment (1.47 *vs.* 1.71 and 1.69kg EE for lambs on the treatments A, I and S respectively, $p<0.05$).

Table 4.16. Analysis of non-carcass components of lambs fed diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (S) for their hourly nutrient release

								Significance		
	BS	BI	BA	SS	SI	SA	s.e.d.	En	Syn	Int
Water (g/kg)	670.5	662.5	676.7	670.6	681.1	692.6	9.82	NS	NS	NS
Ash (g/kg)	22.0	22.5	22.3	33.3	20.2	33.3	6.31	NS	NS	NS
CP (g/kg)	154.3	160.5	160.0	169.7	168.0	166.0	5.53	**	NS	NS
EE (g/kg)	134.6	140.5	121.5	115.1	112.2	101.9	9.80	***	NS	NS
Total DM (kg)	4.51	4.51	4.33	4.38	4.35	4.00	0.171	*	*	NS
Total OM (kg)	4.21	4.21	4.03	3.92	4.08	3.68	0.180	*	NS	NS
Total CP (kg)	2.11	2.14	2.15	2.25	2.29	2.13	0.092	NS	NS	NS
Total EE (kg)	1.85	1.87	1.63	1.53	1.54	1.31	0.135	***	NS	NS

En = Effect due to energy source, Syn = Effect due to synchrony of N and OM supply to the rumen, Int = Interaction effect. * = $p<0.05$, ** = $p<0.005$, *** = $p<0.001$, NS = $p>0.05$.

Lambs on the barley based diet had significantly higher quantities of dry matter ($p<0.05$), organic matter ($p<0.01$) and ether extract ($p<0.001$) deposited in the whole body than lambs on the sugar beet based diets (Table 4.17). Lambs on the asynchronous treatments (BA and SA) tended to deposit less fat in the whole body than lambs on either the synchronous (BS and SS) or intermediate (BI and SI) treatments ($p<0.1$). Within the barley based diets, lambs on the synchronous treatment deposited the most fat in the whole body, and those on the asynchronous treatment deposited the least, although the difference was not statistically significant.

Table 4.17. Whole body composition (kg) of lambs fed diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (S) for their hourly release of nitrogen and organic matter in the rumen.

								Significance		
	BS	BI	BA	SS	SI	SA	s.e.d.	En	Syn	Int
DM	12.42	11.78	11.64	11.35	11.77	10.98	0.366	*	NS	NS
OM	11.18	10.66	10.41	10.10	10.66	9.83	0.338	**	NS	NS
CP	5.44	5.30	5.45	5.44	5.59	5.43	0.162	NS	NS	NS
EE	5.42	5.16	4.64	4.08	4.76	4.04	0.385	**	NS	NS

En = Effect due to energy source, Syn = Effect due to synchrony of N and OM supply to the rumen, Int = Interaction effect. * = $p<0.05$, ** = $p<0.005$, *** = $p<0.001$, NS = $p>0.05$.

There were no significant differences in nitrogen retention due to energy source or synchrony either on a daily basis or in terms of g N retained / g N intake (Table 4.18). There was a tendency however for lambs fed the sugar beet based diets to retain more N on a daily basis than lambs fed the barley based diets (4.03 g/d *vs.* 4.59 g/d for lambs fed the barley and sugar beet based diets respectively. $p = 0.058$)

Lambs fed the barley based diets had significantly higher GE intakes than lambs fed the sugar beet based diet and also retained significantly more GE over the course of the trial (158 MJ *vs.* 127MJ for lambs on the barley and sugar beet based diets respectively) (Table 4.20). Lambs on the asynchronous treatments (BA and SA) retained significantly less GE (MJ) than lambs on either the synchronous (BS and SS) or intermediate (BI and SI) treatments ($p<0.05$). Although there were no significant differences in the quantity of GE retained on a daily basis, there was a tendency for lambs on the barley based diets to deposit more GE per day than lambs on the sugar beet based diets (1.78MJ/d *vs.* 1.58MJ/d for diets B and S respectively, $p=0.084$), and for labs on the asynchronous diets (BA and SA) to deposit less GE per day than lambs on the synchronous or intermediate diets (1.49, 1.80 and 1.76MJ/d for diets A, I and S respectively, $p= 0.059$). Lambs on the barley based diets retained a significantly higher

proportion of energy intake than lambs on the sugar beet based diets (0.095 *vs.* 0.084 MJ/MJ respectively, p= 0.047), and lambs on the asynchronous diets retained a significantly lower proportion of energy intake that lambs on either the synchronous or intermediate diets (0.079 *vs.* 0.097 and 0.092 MJ/MJ for diets A, I and S respectively, p= 0.017).

Table 4.18. Nitrogen retention of lambs fed diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (S) for their hourly release of nitrogen and organic matter in the rumen.

								Significance		
	BS	BI	BA	SS	SI	SA	s.e.d.	En	Syn	Int
N intake (kg)	2.32	2.25	2.27	2.16	2.17	2.22	0.119	NS	NS	NS
Initial N content (g)	638	635	645	666	654	659	17.1	NS	NS	NS
Final N content (g)	997	991	1011	986	1056	966	6702	NS	NS	NS
N deposited (g)	388	356	361	368	402	383	29.2	NS	NS	NS
N retained (g/d)	4.20	3.88	4.01	4.65	4.70	4.41	0.373	NS	NS	NS
N retained (g/g intake)	0.165	0.157	0.160	0.171	0.185	0.169	0.016	NS	NS	NS

En = Effect due to energy source, Syn = Effect due to synchrony of N and OM supply to the rumen, Int = Interaction effect. * = p<0.05, ** = p<0.005, *** = p<0.001, NS = p>0.05.

Table 4.19. Energy retention of lambs fed diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (S) for their hourly release of nitrogen and organic matter in the rumen.

								Significance		
	BS	BI	BA	SS	SI	SA	s.e.d.	En	Syn	Int
GE intake (MJ)	1745	1634	1691	1514	1572	1539	85.6	*	NS	NS
Initial GE content (MJ)	170	169	173	177	174	175	4.56	NS	NS	NS
Final GE content (MJ)	345	328	311	298	319	289	13.4	**	*	NS
Total GE retained (MJ)	176	159	138	122	145	114	13.4	***	*	*
Energy retained (MJ/d)	1.92	1.86	1.56	1.59	1.75	1.41	0.256	NS	NS	NS
Energy retained (MJ/MJ intake)	0.101	0.100	0.083	0.085	0.094	0.075	0.0085	*	*	NS

4.3.3. Rumen Parameters.

4.3.3.1. Rumen pH

Overall mean rumen pH was highest at 08.00h (pH 7.1), and decreased to a mean value of pH 6.4 at 12.30h, before increasing again to pH 6.6 at 15.00h, prior to feeding and decreasing to a minimum of pH 6.3 at 19.30 (Figure 4.2). There was no significant difference between energy sources in rumen pH at any time point. The only significant effect due to synchrony occurred at 19.30h when lambs on synchronous treatments (BS and SS) had a significantly lower rumen pH than lambs on the asynchronous treatments (BA and SA) ($p<0.05$). On analysing the barley and sugar beet diets separately, it was found that the lambs on the synchronous barley treatment had significantly lower rumen pH at this time point than lambs on either of the other two barley diets (pH 5.7 *vs.* 6.5 and 6.7 for treatments BS, BI and BA respectively. $p<0.01$).

4.3.3.2. Rumen ammonia

Although there were no significant differences in mean rumen ammonia concentrations over the total sampling period due to energy source or synchrony, significant differences in rumen ammonia due to energy source were observed at certain time points (Figures 4.3. a and b). At 08.00h lambs on the sugar beet based diets had significantly higher rumen ammonia concentrations than lambs on the barley based diets ($p < 0.005$) whilst at both 12.30 and 17.30h the lambs on the barley based diets had significantly higher rumen ammonia concentrations than lambs on the sugar beet diets ($p < 0.05$).

Significant differences due to synchrony were also seen at certain time points. At 10.30h, lambs on the intermediate treatments (BI and SI) had significantly lower rumen ammonia

concentrations than lambs on either the synchronous (BS and SS) or asynchronous (BA and SA) treatments ($p<0.001$), whilst at 12.30h the lambs on the intermediate treatments had significantly lower rumen ammonia concentrations than lambs on the asynchronous treatment ($p<0.01$). At 17.30h lambs on the asynchronous treatments had significantly lower rumen ammonia concentrations than lambs on either the synchronous or intermediate treatments (mean values of 289.0, 353.0 and 97.5mg/l for the synchronous, intermediate and asynchronous treatments respectively, $p<0.001$), and at 19.30h they were significantly lower than those lambs on the synchronous treatments ($p<0.05$).

4.3.3.3. Rumen VFAs.

The daily variation in total VFA concentrations are presented in Figure 4.4 and the variation in molar proportions of VFA are presented in Table 4.20. There were no significant differences overall in the total VFA concentration due to either energy source or synchrony. Energy source but not synchrony had a significant effect on the molar proportion of acetate, with lambs fed the barley based diets having significantly lower molar proportion of acetate than those fed the sugar beet based diets ($p<0.05$). There were no significant effects on the molar proportion of propionate, whilst lambs fed the barley based diets had significantly higher molar proportions of butyrate than those fed the sugar beet based diets (<0.005). Time had a significant effect on total volatile fatty acid concentration, with the lowest concentration (45mM) being seen at 08.00h, with a gradual increase during the day to the maximum concentration of 73.3mM at 19.30h.

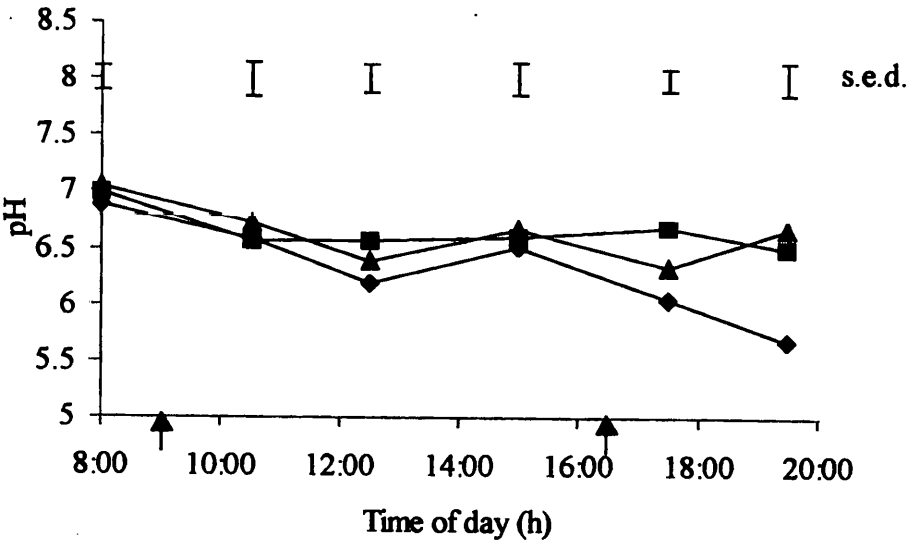
Some differences in VFA concentration due to synchrony were seen at specific time points. At 10.30 lambs on the synchronous treatments had significantly lower molar proportion of

acetate ($p<0.05$) than lambs on the intermediate or asynchronous treatments. On the sugar beet diets, the molar proportion of acetate was significantly lower in lambs on the intermediate treatment than lambs on either of the other two treatments ($p<0.05$), and on the barley diet it was significantly lower in the lambs on the intermediate treatment than in lambs on the asynchronous treatment ($p<0.05$). On the sugar beet diets the molar proportion of propionate was significantly lower in lambs on the intermediate diet than in lambs on either of the other two treatments ($p<0.05$), and lambs on the synchronous treatment had significantly lower molar proportions than lambs on the asynchronous treatment ($p<0.05$). At 15.00h lambs on the intermediate barley treatment had significantly higher molar proportion of acetate then lambs on either of the other two barley treatments ($p<0.05$).

Table 4.20. Molar proportions of rumen volatile fatty acids in *lambs fed diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (S) for their hourly release of nitrogen and organic matter in the rumen.*

	08.00	10.30	12.30	15.00	17.30	19.30
Acetate						
BS	0.623	0.512	0.554	0.570	0.519	0.542
BI	0.658	0.595	0.610	0.628	0.575	0.597
BA	0.648	0.555	0.550	0.570	0.590	0.572
SS	0.630	0.605	0.645	0.653	0.635	0.633
SI	0.605	0.628	0.608	0.603	0.600	0.605
SA	0.625	0.583	0.610	0.618	0.650	0.653
s.e.d.	0.0373	0.0247	0.0305	0.0229	0.0375	0.0350
Propionate						
BS	0.183	0.288	0.231	0.230	0.271	0.285
BI	0.185	0.228	0.218	0.192	0.248	0.230
BA	0.178	0.258	0.240	0.225	0.220	0.223
SS	0.193	0.262	0.245	0.198	0.268	0.232
SI	0.178	0.228	0.255	0.233	0.280	0.277
SA	0.190	0.290	0.255	0.238	0.240	0.250
s.e.d.	0.0233	0.0291	0.0330	0.0311	0.0325	0.0316
Butyrate						
BS	0.143	0.157	0.170	0.160	0.165	0.130
BI	0.115	0.140	0.135	0.133	0.138	0.140
BA	0.128	0.141	0.160	0.153	0.158	0.163
SS	0.110	0.087	0.080	0.108	0.070	0.102
SI	0.141	0.070	0.103	0.118	0.093	0.089
SA	0.108	0.085	0.093	0.100	0.078	0.069
s.e.d.	0.0306	0.0318	0.0326	0.0283	0.0297	0.0286

a)



b)

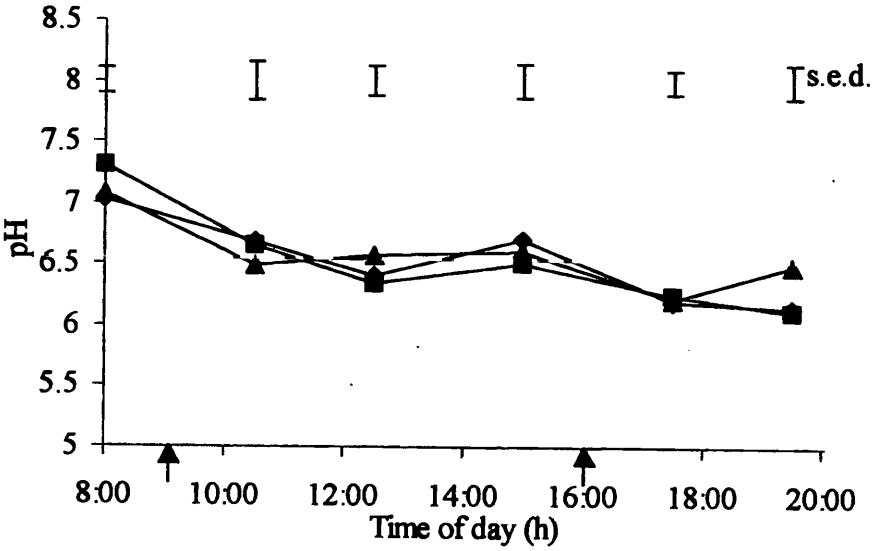
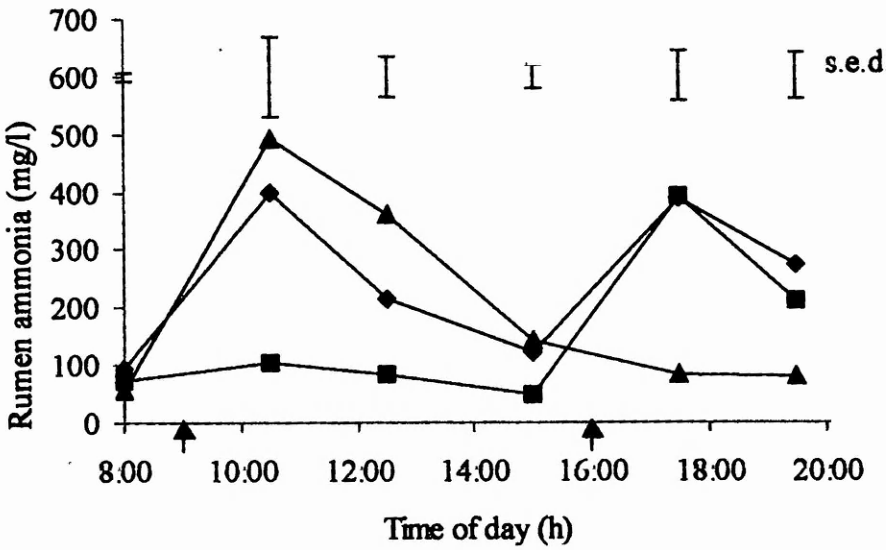


Figure 4.2. Rumen pH in lambs fed a) barley based diets and b) sugar beet based diets differing in sequence of allocation of individual ingredients to be synchronous (◆), intermediate (■) or asynchronous (▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate time of feeding.

a)



b)

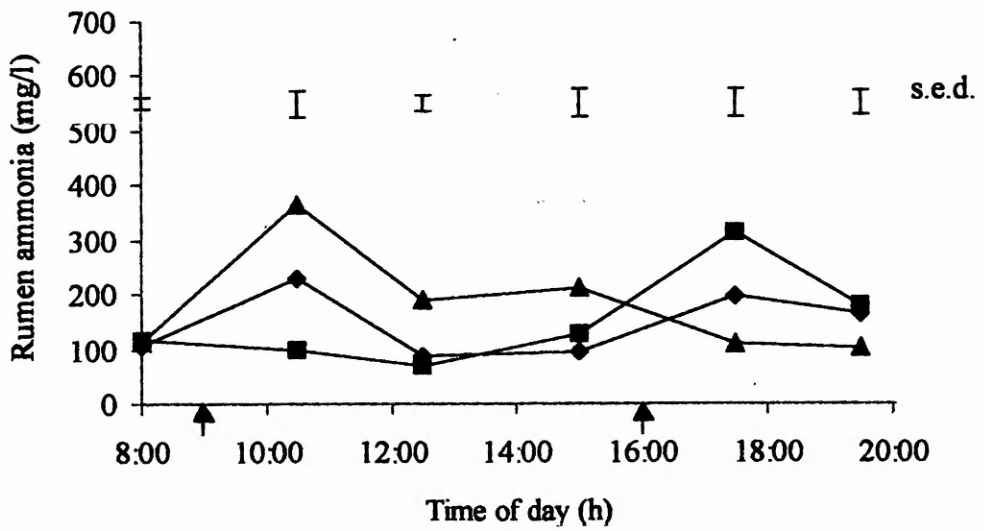
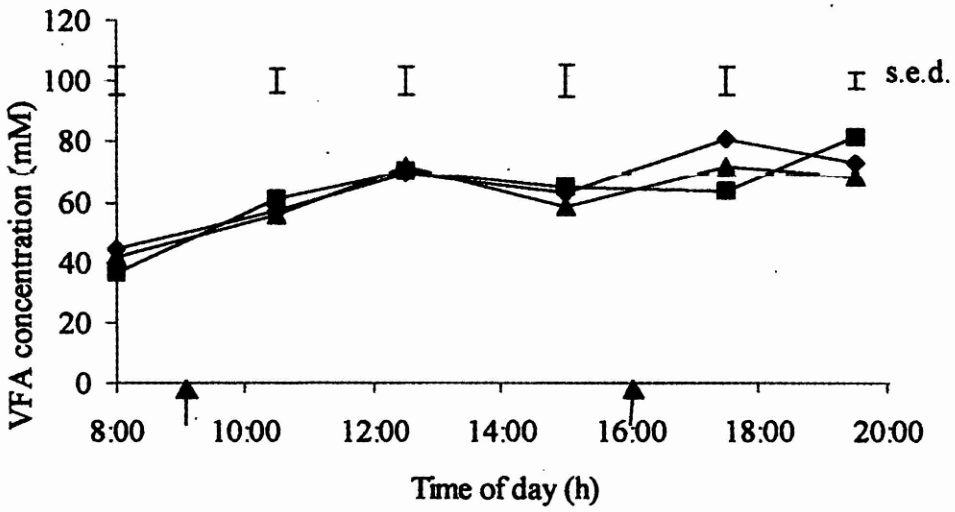


Figure 4.3. Rumen ammonia concentrations in lambs fed a) barley based diets and b) sugar beet based diets differing in sequence of allocation of individual ingredients to be synchronous (◆), intermediate (■) or asynchronous (▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate time of feeding.

a)



b)

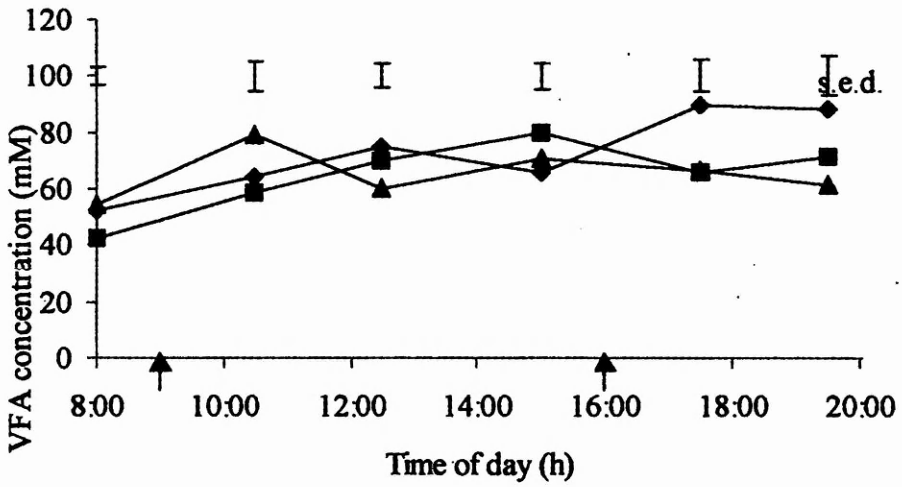


Figure 4.4. Total rumen VFA concentration in lambs fed a) barley based diets and b) sugar beet based diets differing in sequence of allocation of individual ingredients to be synchronous (◆), intermediate (■) or asynchronous (▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate time of feeding.

4.3.4. Blood metabolites.

4.3.4.1. Plasma ammonia

Lambs on all treatments exhibited a cyclical trend in plasma ammonia concentrations (Figure 4.5). Overall lambs on the barley diet exhibited significantly higher plasma ammonia concentrations that those on the sugar beet based diets with mean values of 47.3μM and 28.0μM respectively ($p<0.001$). Significant differences due to synchrony were seen at 10.30h and 12.30h, when lambs on asynchronous treatments (BA and SA) had significantly higher plasma ammonia concentrations than those on intermediate treatments ($p<0.001$). At 17.30h lambs on intermediate treatments produced significantly higher plasma ammonia concentrations than lambs on synchronous or asynchronous treatments ($p<0.01$).

Within the barley based diet there were significant peaks in plasma ammonia at 10.30h and 17.30h. Significant differences due to synchrony were seen in the post feeding periods, i.e. at 10.30h and 12.30h when lambs on treatment BA had significantly higher plasma ammonia concentrations than those on treatment BI ($p<0.05$), and at 17.30h when treatment BI produced significantly higher plasma ammonia concentrations than treatments BA or BS ($p<0.05$).

Within the sugar beet based diet a slightly different pattern of plasma ammonia concentrations was seen, with low concentrations (<40μM) throughout the day, and no major peaks. The highest plasma ammonia concentration of 39.8μM seen at 08.00h with a gradual decline throughout the day to the lowest value at 22.00h. The only significant difference due to synchrony was seen at 12.30h when lambs on the synchronous diet (SS) had significantly lower levels of plasma ammonia than lambs on the other two treatments with mean values of 23.2μM,

29.0μM and 29.5μM for treatments SS, SI and SA respectively.

4.3.4.2. Plasma urea.

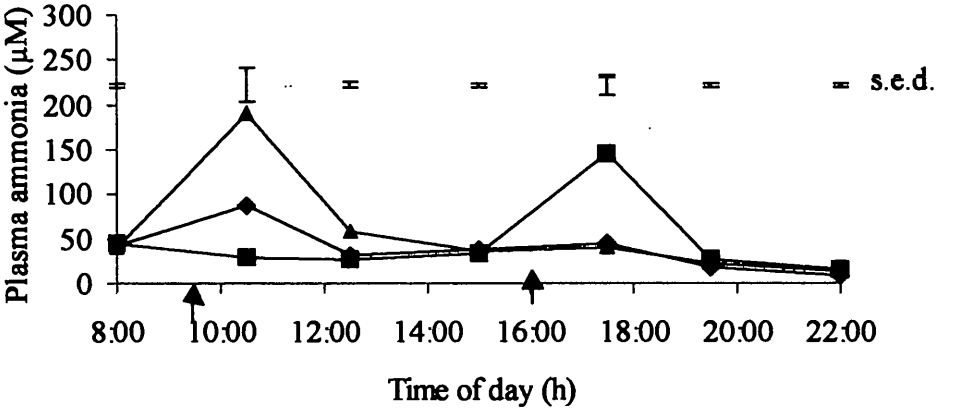
The daily variations in plasma urea concentrations are presented in Figure 4.6. Overall there were no significant differences between treatments due to either energy source or synchrony. However there was a significant effect of time ($p<0.001$), and a significant interaction between time and synchrony ($p<0.001$).

Within the barley based diets, lambs on the asynchronous treatment (BA) had significantly lower plasma urea concentrations at 08.00h than lambs on either the intermediate (BI) or synchronous (BS) treatments (mean values of 5.9mM, 6.2mM and 4.2mM for synchronous, intermediate and asynchronous treatments respectively, $p<0.001$), whilst at 10.30h, lambs on both the asynchronous and intermediate treatments had significantly lower levels than lambs on the synchronous treatment ($p<0.05$). At 12.30h and 15.00h lambs on the intermediate treatments had significantly lower levels of plasma urea than lambs on the synchronous or asynchronous treatments ($p<0.001$), whilst at 15.00h lambs on the synchronous treatments had significantly lower plasma urea concentrations than lambs on the asynchronous treatments ($p<0.001$). At 22.00h, lambs on the asynchronous treatment had significantly lower plasma urea concentrations than lambs on the intermediate diets ($p<0.001$).

Within the sugar beet diets lambs on the asynchronous treatment (SA) tended to have lower plasma urea concentrations at 08.00h than lambs on treatments SI or SS ($p=0.05$). A 10.30h there was no significant difference between treatments, whilst at 12.30h lambs on treatment SI had significantly lower plasma urea concentrations than lambs on treatments SA or SS (5.4mM

vs. 7.15mM and 7.06mM for treatments SI, SA and SS respectively; $p<0.05$). At 15.00h lambs on the asynchronous treatment (SA) had significantly higher plasma urea concentrations than lambs on either the synchronous (SS) or intermediate (SI) treatments ($p<0.01$), whilst at 17.30h lambs on the synchronous diet had significantly lower plasma urea than lambs on either of the other two treatments (5.12mM *vs.* 6.8mM and 6.46mM for treatments SS, SA and SI respectively; $p<0.05$). At 19.30h lambs on treatment SI had significantly higher plasma urea concentrations than lambs on either treatment SA or SS ($p<0.05$), whilst at 22.00h there were no significant differences in plasma urea concentrations.

a)



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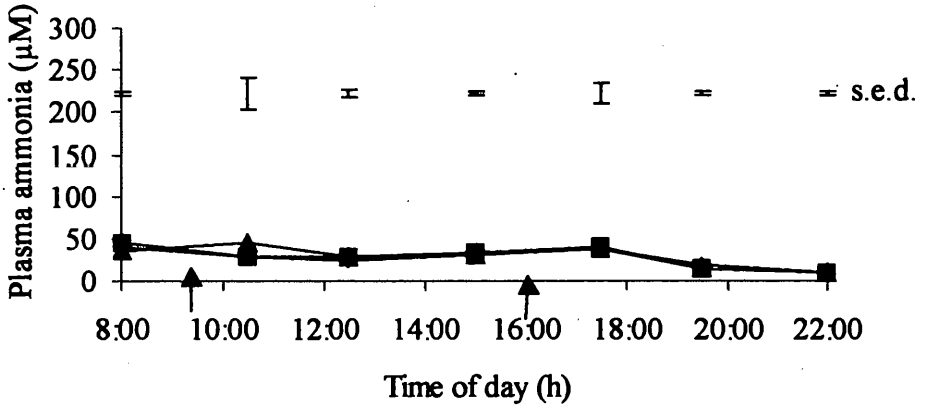
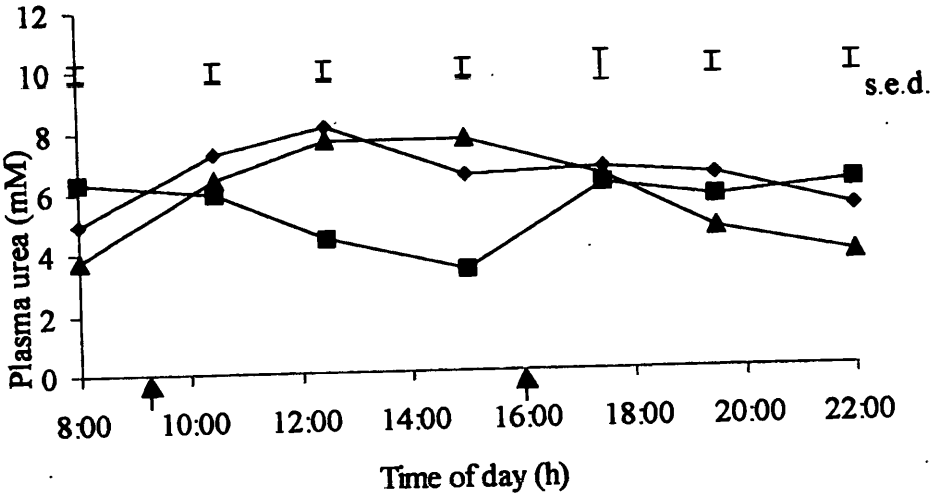


Figure 4.5. Plasma ammonia concentrations in lambs fed either a) barley based diets or b) sugar beet based diets differing in sequence of allocation of individual ingredients to be synchronous(◆), intermediate(■) or asynchronous(▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate time of feeding.

a)



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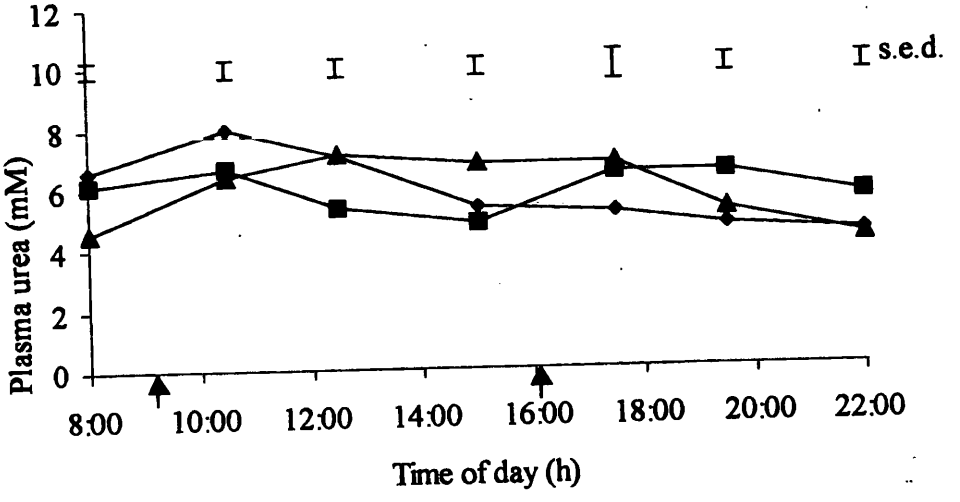


Figure 4.6. Plasma urea concentrations in lambs fed a) barley based diets and b) sugar beet based diets differing in sequence of allocation of individual ingredients to be synchronous(◆), intermediate(■) or asynchronous(▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate time of feeding.

4.3.4.3. Plasma β-hydroxy butyrate.

There were no overall effects of synchrony or energy source on plasma β-hydroxybutyrate concentrations, however there was a significant effect of time ($p<0.01$) (Figure 4.7). On both energy sources the lowest concentration was seen at 08.00h (mean value 0.28mM) with the maximum concentration occurring at 12.30h (0.68mM) followed by a gradual decrease throughout the day. Although there was no overall effect of energy source, significant differences were seen at 12.30h and 15.00h when lambs on the barley based diets had higher plasma concentrations of β-hydroxybutyrate than lambs on the sugar beet based diets ($p<0.05$).

4.3.4.4. Plasma glucose.

Overall there were no significant differences in plasma glucose concentration caused by either energy source or degree of synchrony but there was a significant effect due to time ($p<0.001$), and a significant interaction between time and synchrony ($p<0.05$) (Figure 4.8). In the lambs fed the synchronous diets (BS and SS) the lowest mean plasma glucose concentration was seen at 10.30h, followed by a peak at 12.30h, a gradual decline until 17.30h and a second larger peak at 19.30h. Lambs on the intermediate treatments (BI and SI) showed a similar pattern of plasma glucose concentration, with the exception that the maximum concentration occurred at 22.00h, whilst those on the asynchronous treatments (BA and SA) had the lowest concentration of plasma glucose at 10.30h, with an overall increase throughout the day to a maximum at 19.30h.

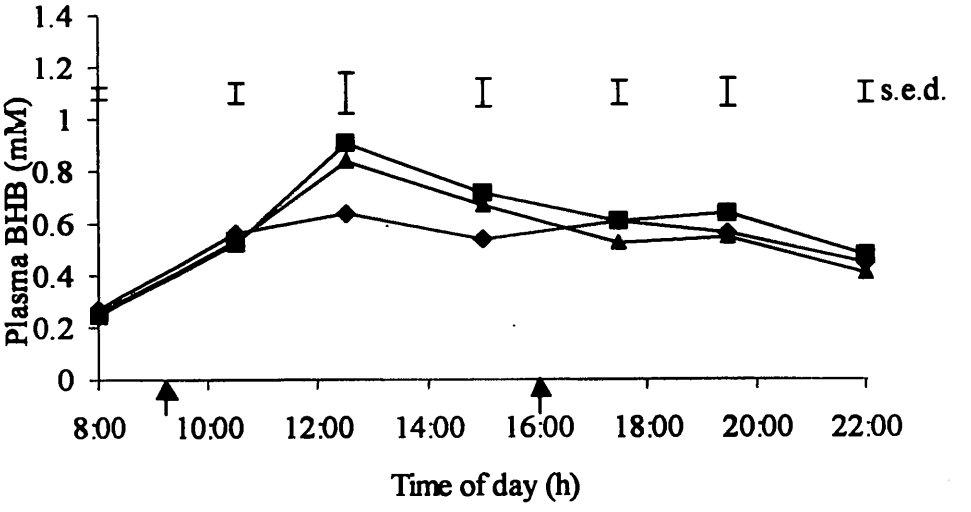
There was a significant interaction between energy source and synchrony at 10.30h ($p<0.05$).

Within lambs fed the barley based diets the synchronous treatment (BS) resulted in the lowest plasma concentration of glucose and the asynchronous treatment (BA) resulted in the highest, with mean values of 4.3mM and 4.7mM respectively. On the sugar beet diets the asynchronous (SA) treatment resulted in the lowest plasma glucose concentration and the intermediate (SI) treatment resulted in the highest (4.2mM *vs.* 4.5mM).

4.3.4.5. Plasma insulin.

Overall there were no significant differences between energy sources or degree of synchrony, however there was a significant effect of time ($p<0.001$) (Figure 4.9). The lowest mean plasma insulin concentration was seen at 08.00h, followed by an increase post feeding, then a decrease until 15.00h, with a second peak occurring post feeding, at 17.30h.

a)



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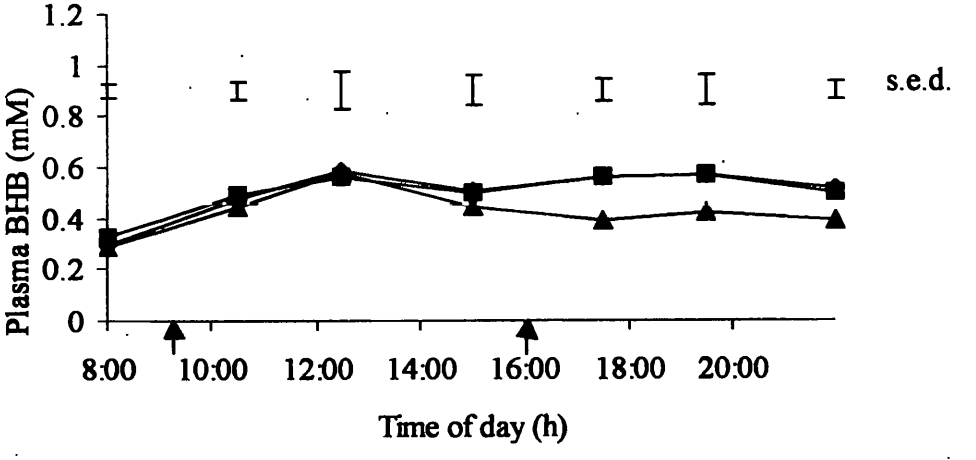
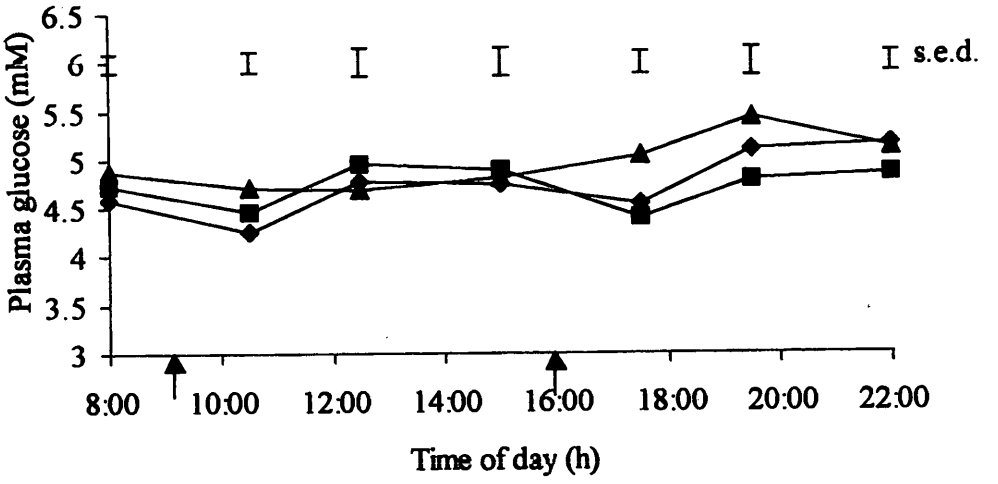


Figure 4.7. Plasma β-hydroxybutyrate (BHB) concentrations in lambs fed a) barley based diets and b) sugar beet based diets differing in sequence of allocation of individual ingredients to be synchronous(◆), intermediate(■) or asynchronous(▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate time of feeding.

a)



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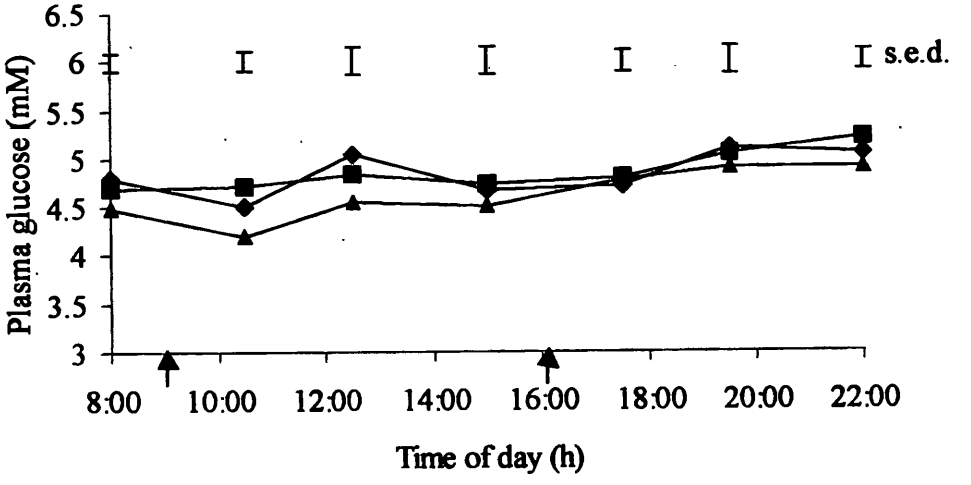
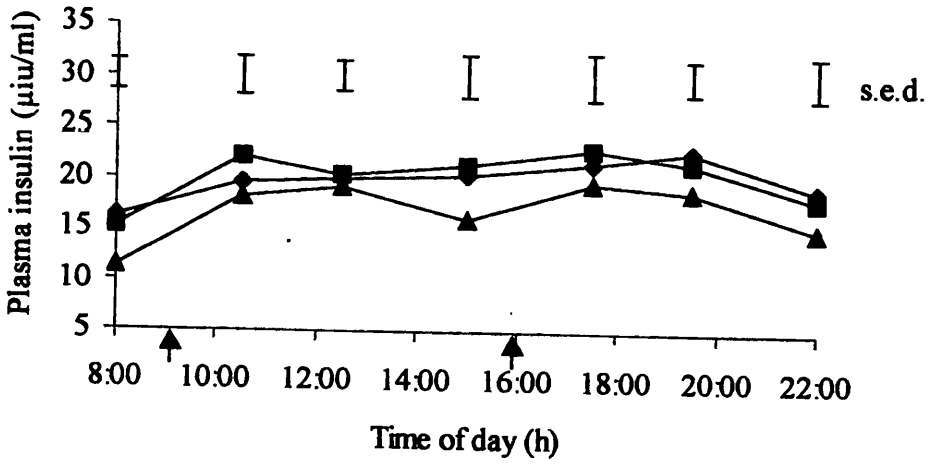


Figure 4.8. Plasma glucose concentrations in lambs on a) barley based diets and b) sugar beet based diets differing in sequence of allocation of individual ingredients to be synchronous(◆), intermediate(■) or asynchronous(▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate time of feeding.

a)



b)

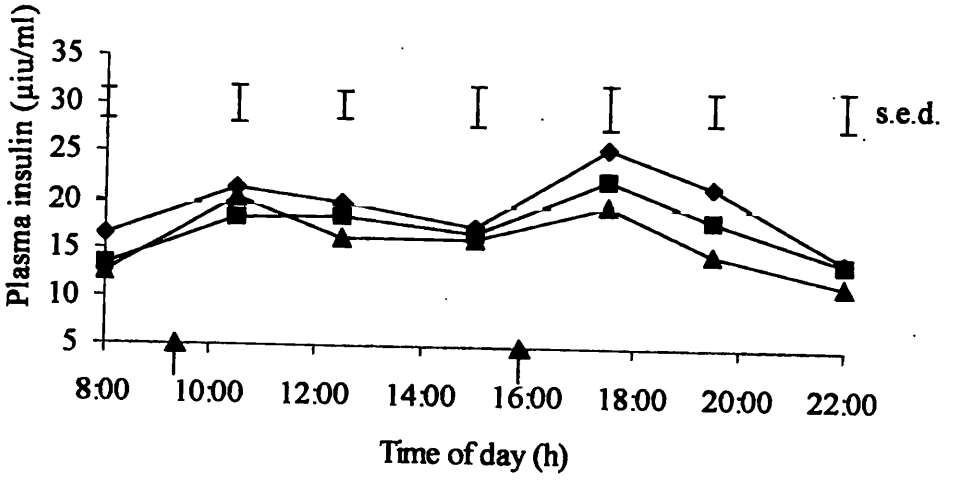


Figure 4.9. Plasma insulin concentrations in lambs fed a) barley based diets and b) sugar beet based diets differing in sequence of allocation of individual ingredients to be synchronous (◆), intermediate(■) or asynchronous(▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate time of feeding.

4.4. Discussion

4.4.1. Feed analysis and nutrient intake

Analysis of the experimental diets showed that the nutrient supply was close to that predicted. The barley based diets were higher in OM, EE and gross energy (GE) than the sugar beet based diets, and lower in NDF.

4.4.2. Growth and carcass characteristics

The mean growth rate in the current experiment was 186.0 g/d, higher than the predicted 175g/d. This may have been due to the lambs being individually penned, and therefore having a slightly lower activity allowance. The calculation of activity allowance recommended by AFRC (1993) for housed fattening lambs assumes that the lamb will walk 50 metres per day and stand for 12 hours. Observation of the lambs in the current experiment showed that they spent the majority of their time lying down, therefore their energy expenditure may be assumed to be less.

Witt *et al.* (1999b) reported significantly greater growth rates and improved feed conversion efficiencies in lambs fed at a restricted level, diets formulated to be synchronous and based on either barley or sugar beet. However, in the current trial such differences were not apparent. In the trial by Witt *et al.* (1999b) both synchronous diets contained a proportion of barley, whilst the two asynchronous diets did not contain any. It has been suggested that energy source within the diet has a greater effect on microbial protein production than synchrony (Henning *et al.*, 1991, 1993; Herrera-Saldana *et al.*, 1990), but there have been few studies investigating whether the difference is translated into differences in growth. If the differences seen by Witt

et al. (1999b) were due to the barley content of the two synchronous diets, rather than to synchrony, it might be expected that lambs on the barley-based diets would grow faster than lambs on the sugar beet-based diets in the current experiment. However this was not the case, with the barley-fed lambs taking significantly longer to reach 40kg live weight than the sugar beet-fed lambs. This appears to support the results of Normand *et al.* (1999) who reported higher growth rates in sugar beet-fed lambs than in wheat and barley fed lambs but contradicts the results of Witt *et al.* (1999a) who reported that when fed *ad libitum*, lambs offered diets with a rapid rate of energy release had significantly higher growth rates than those offered diets with a slow rate of energy release. Mandebvu and Galbraith (1999) reported no differences in live weight gain or feed conversion efficiency in lambs fed either barley based or sugar beet based diets.

The lack of an effect of carbohydrate source or synchrony on carcass characteristics seen in the current experiment is in agreement with Witt *et al* (1999b). Lambs on synchronous treatments deposited significantly more kidney knob and channel fat, a result consistent with those of Witt *et al.* (1999b). However in the current study energy source within the diet had a significant effect on kidney fat, with lambs on the rapidly degradable energy source (barley) depositing significantly more, a result which is in agreement with those of Normand *et al.* (1999) who reported significantly greater internal fat deposition in cereal-fed lambs than in sugar beet-fed lambs, and Mandebvu and Galbraith (1999) who reported increased perirenal fat deposition with increased proportion of barley :sugar beet within the diet. The diets used by Witt *et al.* (1999b) were based on a variety of energy sources (barley, sugar beet pulp and tapioca) and it is therefore possible that any potential effects due to synchrony were confounded by the presence of a variety of energy sources within the same diet. In the current experiment the increase in kidney knob and channel fat in lambs fed the synchronous diets was seen on both

energy sources, which indicates that it is a real effect of synchrony.

It appears that energy source within the diet had a greater effect on body composition than synchrony, with lambs on the barley based diets depositing significantly more dry matter, organic matter and fat in the whole body than lambs on the sugar beet based diets. However, protein deposition was not affected. The greater fat deposition may be due to the observed difference in GE intakes between the two energy sources. The lack of significant differences in daily energy retention between the two energy sources, despite the differences in total energy retention are a reflection of the fact that either energy intake or the number of days on trial were a factor in the calculation of energy balance. As these were both significantly higher in lambs on the barley based diets, the calculated energy balances were similar between energy sources (mean values of 2.0MJ/d or 0.1MJ retained / MJ intake and 1.9MJ/d or 0.1MJ retained / MJ intake for lambs on barley and sugar beet based diets respectively). Mandebvu and Galbraith (1999) reported that increasing the proportion of barley : sugarbeet pulp in the diet resulted in an increase in the proportion of crude protein in the live weight gain of growing lambs, but did not affect the proportion of fat in the gain, a result which would seem to contradict those of the current experiment.

Within energy source however there were no differences in nutrient intake. The lower fat deposition in lambs on the asynchronous diets therefore suggests a decreased efficiency of energy utilisation in these lambs, which is reflected in the calculated energy retention. The mean calculated efficiency of energy utilisation in the current experiment (0.102 MJ retained/MJ intake) is much lower than that reported by Wilkinson and Greenhalgh (1995) (0.283MJ retained/MJ intake). The lambs used by Wilkinson and Greenhalgh (1995) were an earlier maturing breed, slaughtered at a higher live weight than the lambs used in the current

experiment, therefore they would be expected to have a higher total body fat content. The efficiency of energy utilisation for deposition and maintenance of fat is greater than that of protein (Ørskov and McDonald, 1970; Pullar and Webster, 1977), therefore the efficiency of energy retention could be expected to be greater. Also the lambs used by Wilkinson and Greenhalgh (1995) were fed *ad libitum*, and therefore had a higher daily live weight gain, resulting in fewer days on trial and consequently a lower total maintenance requirement.

The lower gut fill seen in the barley fed lambs compared to the sugar beet fed lambs may be due to the diet being more digestible due to its higher content of rapidly degradable OM as determined in the previous experiment (Chapter 3). The results from Chapter 3 show that the barley used in these diets had a much greater rapidly degradable fraction (*a*) than the sugar beet, and the rate of degradation (*c*) of the potentially degradable fraction (*b*) was much greater in the barley than the sugar beet, a result in agreement with those reported by Witt *et al.*, (1999a). Retention time in the rumen is highly correlated with level of feed intake (AFRC, 1993), therefore as all treatments received the same quantity of feed DM, it can be assumed that outflow rates from the rumen similar were similar. Therefore a higher proportion of the barley diets would be degraded, utilised by the rumen microbes and absorbed by the animal, resulting in a lower proportion of the diet remaining in the GI tract.

4.4.3. Rumen parameters.

4.4.3.1. Rumen pH

The optimum pH for cellulolytic activity is approximately pH6.8 (McCarthy *et al.*, 1989). The observed pH range in this experiment was pH6.7 – 7.3, and therefore cellulolytic activity should

have been at or close to maximum on all treatments. In general diets containing a high proportion of rapidly fermentable carbohydrate, such as barley, would be expected to result in a lowering of rumen pH (Moss *et al.*, 1995) however in the current experiment there was no difference between energy sources in total concentrations of VFA at any time, a fact reflected in the lack of any significant differences in rumen pH.

4.4.3.2. Rumen ammonia

Rumen ammonia concentration is a function of the rate of release of dietary N, the rate at which it can be taken up and utilised by the rumen microbes and the rate of absorption through the rumen wall (Nolan, 1993). The rate of microbial utilisation of ammonia is affected by the availability of fermentable ME (FME) (AFRC 1995). Within energy source, all lambs received similar quantities of degradable OM in each meal, therefore in this trial it would be expected that the rate of N release would be the major factor influencing rumen ammonia concentrations (Sinclair *et al.*, 1993, 1995). Indeed this does appear to be the case, with those lambs receiving the greatest quantity of dietary protein in a particular meal showing the highest rumen ammonia concentrations post feeding. Although not statistically significant, the concentrations of ammonia in the rumen were higher in those lambs on the barley diets. This may have been due to the barley diet containing a higher proportion of the more rapidly degraded protein sources (urea and distillers grains) than the sugar beet diet. Mandebvu and Galbraith (1999) also reported increased rumen ammonia concentrations with increasing proportion of barley:sugar beet pulp in the diet. High propionate concentrations are known to have a detrimental effect on the metabolism of ammonia post absorption leading to increased plasma ammonia concentrations (Choung and Chamberlain, 1995) although there seems to be a lack of information on whether high propionate producing diets might have a similar effect on

metabolism of ammonia by rumen microbes. In the current experiment there were no differences between treatments in rumen propionate concentrations, therefore it is unlikely to have been a factor in the difference in rumen ammonia concentration between energy sources.

At no point did rumen ammonia concentrations fall below the value of 50mg/l quoted by Satter and Slyter (1974) as being the minimum required for the maximum efficiency of microbial growth. It would therefore be expected that none of the treatments would have adversely affected microbial activity.

4.4.3.3. Rumen VFAs

The concentration of VFAs in the rumen is determined by the relative rates of production, absorption and outflow rate (Leng and Brett, 1966; Bergman, 1990; Dijkstra *et al.*, 1993). Leng and Brett (1966) reported that the concentration of VFA in the rumen is well correlated with the rate of production. The rate of absorption of VFA and thus their clearance rate from the rumen is affected by a number of factors, including VFA concentration, rumen pH and the volume of liquid in the rumen (Dijkstra *et al.*, 1993). Dijkstra *et al.* (1993) reported that in lactating dairy cows fractional absorption rates of acetate, propionate and butyrate were reduced by increasing rumen fluid pH, and by increasing rumen fluid volume. Increasing VFA concentration however had varying effects, depending on which VFA was under consideration. Increasing the concentration of acetic acid increased fractional absorption rate of acetic acid, whilst increasing the concentration of propionic acid tended to decrease the fractional absorption rate. The fractional absorption rate of butyric acid was unaffected by concentration. This would indicate that the final concentrations of VFA in the rumen are not in fact as clear an indicator of rate of production as was postulated by Leng and Brett (1966). However the

concentration of total VFAs used in the experiment by Djikstra *et al.* (1993) was 170mM, which is higher than might be expected under normal feeding conditions. In contrast the concentrations reported by Leng and Brett (1966) ranged from 56 to 172mM, which are closer to the normal range of 70 to 130mM quoted by France and Siddons (1993). The overall mean VFA concentrations in the current experiment of 45-75mM were generally lower than those quoted by France and Siddons (1993) and may reflect the fact that the animals were fed at a restricted level. Witt *et al.* (1999) fed growing lambs at a restricted level similar to that used in the current experiment and reported total VFA concentration ranging from 24 to 48mM.

In the current experiment there were no significant differences between treatments in rumen fluid pH, and as all lambs were fed at the same restricted level it is likely that there were no significant differences in rumen fluid volume or outflow rate. Therefore the major determinant of the pattern of VFA absorption would have been the pattern of production. The pattern of absorbed VFA (i.e. the relative amounts of the glucogenic propionate and the lipogenic acetate and butyrate) affects the efficiency of VFA utilisation (MacRae and Lobley, 1982). The overall molar proportions of acetate:propionate:butyrate were 58:28:14 on the barley based diets and 62:28:10 on the sugar beet diet which are within the normal range quoted by Bergman (1990). The overall fermentation pattern was significantly different between energy sources with barley diets producing lower molar proportions of acetate and higher molar proportions of butyrate than the sugar beet diets. Generally diets containing a source of starch, such as cereal grains, result in increased propionate production and decreased acetate production (Bergman, 1990). Witt *et al.* (1999a,b) and Mandebvu and Galbraith (1999) reported significantly higher rumen concentrations of propionate and significantly lower concentrations of acetate in sheep fed diets containing rapidly degradable energy sources (barley or tapioca) than in sheep fed slowly degradable energy sources (sugar beet pulp). However Thorp *et al.* (2000) reported increased

rumen butyrate concentrations with increasing inclusion of barley in a silage based diet, as did Wylie *et al.* (1983), which would seen to concur with the results from the present experiment.

The proportion of propionate utilised for glucose synthesis is relatively constant under various physiological and nutritional conditions (Brockman, 1993). As propionate concentrations in the rumen were not significantly different between treatments it is probable that the supply of glucose to the animal was similar on all treatments. MacRae and Lobley (1982) suggested that the lower efficiency of energy utilisation seen in roughage-fed animals compared to concentrate-fed animals may be due to an inability to handle excess acetate. The conversion of acetate to fat requires the presence of $NADPH_2$, which in turn requires adequate propionate and hence glucose supply. In the absence of adequate $NADPH_2$ acetate has to be eliminated via some form of futile cycle, resulting in loss of energy as heat (MacRae and Lobley, 1982). However in the current experiment the potential for glucose production from propionate, and therefore $NADPH_2$ supply from this source appears to have been similar on all treatments, therefore the potential for fat deposition should have been similar. Therefore the difference between energy sources in fat deposition may have arisen from some process other than VFA metabolism.

Within energy sources there were no significant differences in molar ratios of volatile fatty acids, which would suggest that there was little difference between treatments in the relative amounts of glucogenic and lipogenic VFA available for absorption. Therefore the efficiency of utilisation of the VFA should have been similar across treatments, so the differences in energy metabolism between animals fed synchronous and asynchronous diets may have been due to some other metabolic process.

4.4.4. Plasma metabolites

4.4.4.1. Plasma ammonia

Ammonia produced in the rumen which exceeds the ability of the rumen microbes to absorb and utilise it in the production of microbial protein is absorbed across the rumen wall, and converted in the liver to urea (Lobley *et al.*, 1995). Thus the concentration of ammonia in the blood is dependant on both ammonia production in the rumen, and the capacity of the liver for conversion.

In the lambs on the barley based diets the pattern of blood ammonia reflects the pattern of rumen ammonia concentration. The fact that such variable levels of ammonia are to be found in the peripheral blood indicates that the quantity of ammonia being absorbed from the rumen exceeded the capacity of the liver to convert in to urea. Figure 4.2a illustrates the fact that rumen ammonia concentrations did reach very high levels (up to 500mg/l) in some animals immediately post feeding. These levels are reflected in high plasma ammonia concentrations of up to 460μM. Orzechowski *et al.* (1987) reported that increasing mesenteric infusion of NH_4Cl from 20μM/min/kg to 30μM/min/kg caused little increase in hepatic uptake of ammonia (1.27μmol/min/g *vs.* 1.45μmol/min/g respectively), but significant increases in ammonia concentration in peripheral blood (139 vs. 500μM) were reported. Linzell *et al.* (1971) reported a maximum rate of hepatic ammonia uptake in isolated perfused ovine livers of 1.18μmol/min/g wet tissue. The maximum ammonia uptake of bovine liver reported by Symonds *et al.* (1981) was 2.6 μM/min/g wet tissue and this occurred at mesenteric infusion rates of between 16 and 19mmol NH_3/min. Above this level, significant increases were seen in carotid and hepatic plasma concentrations of ammonia. The above studies have all used plasma

N metabolite concentrations and hepatic blood flow measurements to determine the rates of hepatic NH_3 absorption and urea production and are in broad agreement that the liver has a limited capacity for ammonia uptake and detoxification, and when ammonia supply from the portal drained viscera (PDV) exceeds this level, peripheral plasma ammonia concentrations will rise. Elevated plasma ammonia has been associated with alterations in glucose metabolism and in insulin and glucagon secretion in cattle (Fernandez *et al.*, 1988, 1990) and sheep (Orzechowski *et al*, 1988) and effects on metabolite and hormone fluxes may occur at levels of ammonia concentration which are below those at which the signs of clinical toxicity are seen (Fernandez *et al.* 1990; Choung and Chamberlain, 1995). There is also evidence that ammonia toxicity may have detrimental effects on reproductive performance (Laven and Drew, 1999) and feed intake (Conrad *et al.* 1977). In the current study all sheep consumed their feed within 30 minutes of feeding, indicating that plasma ammonia concentrations were not reaching the levels necessary to depress feed intake.

The detoxification of ammonia by the liver has been shown to require an input of amino acid N, and to increase the energy expenditure of the liver (van der Walt, 1993; Lobley *et al.*, 1995). Therefore it is possible that lambs experiencing high plasma ammonia concentrations may have had less amino acids and metabolisable energy available for productive purposes.

4.4.4.2. Plasma urea

In ruminants urea is formed in the liver from ammonia which is either absorbed from the gastro-intestinal tract, or arises as a result of amino acid or nucleic acid catabolism (Nolan, 1993). The ammonia removed by the liver from the portal vein provides only one N atom to urea, the other being supplied by cytoplasmic aspartate (Lobley *et al.*, 1995). The N donor to aspartate is an

amino acid, probably glutamate (Lobley *et al.*, 1995). Thus detoxification of large amounts of plasma ammonia, as seen in the asynchronous barley treatment employed in the present study (BA) would be expected to require an input of amino acids, rendering them unavailable for productive purposes. In the current study the concentrations of plasma urea were similar, and followed similar patterns within both energy sources. The range of plasma urea concentrations seen in the present experiment is similar to those reported by Witt *et al.* (1999a,b) and Mandebvu and Galbraith (1999) who also reported a cyclical pattern of plasma ammonia concentration with peaks occurring within 3 hours of feeding. Mandebvu and Galbraith (1999) reported increased plasma urea concentrations with increasing proportions of barley: sugar beet pulp in the diet which mirrored the increases in rumen ammonia seen.

From figures 4.3, 4.7 and 4.8 it is apparent that for the lambs fed the barley based diet, plasma ammonia concentration provided a more accurate picture of rumen ammonia concentration than did plasma urea. However on the sugar beet based diets neither plasma metabolite followed the pattern of rumen ammonia concentration very closely.

4.4.4.3. Plasma β-hydroxy butyrate

Plasma β-hydroxybutyrate is generally used as an indicator of energy metabolism and arises from two main sources in ruminants. The first is hepatic ketogenesis of free fatty acids mobilised from the adipose tissue, and the second is from alimentary ketogenesis from absorbed butyrate in the rumen wall (Brockman, 1993). Ketogenesis from adipose tissue generally occurs in situations where the animal is under supplied with carbohydrate and is in negative energy balance. An insufficient supply of carbohydrate leads to a lack of glucose supply, which in turn leads to a lack of availability of oxaloacetate with which acetyl Co A combines to enter the

TCA cycle (Stryer, 1988). In this situation acetyl Co A is converted to β-hydroxybutyrate. Therefore it may be expected that after feeding β-hydroxybutyrate levels would decrease due to an increase in the availability of glucose, and hence oxaloacetate. In the current experiment it is unlikely that the lambs were mobilising body fat as they were being fed at 2 x maintenance and were growing throughout the trial. Therefore any fluctuations in plasma β-hydroxybutyrate are likely to have been as a result of fluctuations in butyrate production in the rumen, and therefore in absorbed β-hydroxybutyrate. Indeed the higher concentrations of plasma β-hydroxybutyrate observed in the barley fed lambs may be a reflection of the higher rumen butyrate production in these lambs, although significant differences in plasma β-hydroxybutyrate were seen at only two time points. Thorp *et al.* (2000) reported significantly higher plasma β-hydroxybutyrate concentrations with increasing inclusion of barley in the diets of steers, and suggested that this was due to the higher concentrations and molar proportions of rumen butyrate generated by these diets.

Post feeding increases in plasma β-hydroxybutyrate have been observed in restricted fed lambs (Witt *et al.*, 1999b; Carro *et al.*, 1994) and steers (Thorp *et al.*, 2000). In the current experiment greater post feeding increases were seen on the intermediate barley and asynchronous barley diets than on the synchronous barley diet which is in agreement with the findings of Witt *et al.* (1999b) who found that lambs on an asynchronous diet containing a rapidly degradable energy source had the highest plasma concentrations of β-hydroxybutyrate. However in the current experiment the post feeding increase was seen only after the morning feed.

4.4.4.4. Plasma glucose

The plasma glucose concentrations in lambs fed the barley based diets appeared to mirror the pattern seen in plasma β-hydroxybutyrate concentrations in that after the morning feed levels were increased in lambs on the asynchronous and intermediate treatments, although these differences were not significant. This is in agreement with Carro *et al.* (1994) who reported a post feeding increase in plasma glucose levels in restricted fed lambs. A post feeding increase in glucose availability would usually be expected to result in a decrease in plasma β-hydroxybutyrate concentration. However in the current experiment, as in a number of previous studies with growing lambs that have reported plasma β-hydroxybutyrate (Witt *et al.*, 1999b; Carro *et al.*, 1994), both glucose and β-hydroxybutyrate increased post feeding.

4.4.4.5. Plasma insulin

Insulin is secreted in response to raised plasma concentrations of glucose, and would therefore be expected to increase post feeding (Grovum, 1995; Linington *et al.*, 1998). In ruminants the conversion of propionate to glucose provides up to 75% of the glucose supply, (Bergman, 1990) therefore it might be expected that lambs on a diet which promotes production of a higher molar proportion of propionate would exhibit higher plasma concentrations of insulin (Grovum, 1995). It is widely accepted that plasma VFA concentrations, particularly that of propionate also have a major influence on plasma insulin (Sutton *et al.*, 1986; Bergman, 1990). However in the current experiment there were no significant differences between lambs fed either of the two energy sources in the quantity or molar proportion of propionate in the rumen, and this was reflected in the lack of significant differences in both plasma glucose and insulin

levels. Galbraith *et al.* (1988) reported higher plasma insulin concentrations in lambs fed barley-based diets than in lambs fed sugar beet-based diets. Conversely, Mandebvu and Galbraith (1999) found no evidence of a difference in plasma insulin concentrations on a diet containing sugar beet pulp as the major energy source compared to a diet containing barley as the major energy source, a result in agreement with those of the current experiment.

Plasma ammonia concentrations may also influence insulin secretion, with increased peripheral ammonia concentrations causing decreased insulin secretion (Choung and Chamberlain, 1995). In this case it might have been expected that sheep on the asynchronous diets, who did experience transient increases in plasma ammonia, may have exhibited a decrease in plasma insulin concentrations. However this was not the case. The reasons for the lack of any effect of either synchrony or energy source on plasma insulin concentrations in the current experiment are not clear.

4.5. Conclusions

In the current experiment, energy source within the diet had a greater effect on metabolism and carcass composition than synchrony with lambs fed barley-based diets depositing more fat in the whole body than lambs fed sugar beet-based diets. Feeding lambs diets formulated to be synchronous in the hourly release of N and OM in the rumen had no effect on growth rate or FCE. However lambs on the asynchronous diets deposited less fat in the carcass than lambs fed synchronous or intermediate diets. Plasma metabolites generally followed a cyclical trend between meals, with plasma ammonia providing a more accurate picture of ammonia production in the rumen than plasma urea in the lambs fed barley based diets.

CHAPTER 5.

THE EFFECT OF SEQUENCE OF CONCENTRATE ALLOCATION ON DIET DIGESTIBILITY AND RUMEN MICROBIAL NITROGEN PRODUCTION IN GROWING LAMBS.

5.1. INTRODUCTION

The study described in Chapter 4 indicates that both energy source within the diet and the degree of synchrony of organic matter (OM) and nitrogen (N) supply to the rumen may result in differences in the efficiency of energy utilisation. These differences may be attributed to a number of factors, including effects on microbial N production, diet digestibility or the efficiency of nutrient utilisation post absorption.

The objectives of this experiment were to investigate whether the differences in energy metabolism seen in the previous experiment may be related to differences in diet digestibility or the efficiency of microbial protein production.

5.2. MATERIALS AND METHODS

5.2.1. Experimental Animals

Twenty four entire male Friesland lambs with an initial live weight of 28kg (s.d. 2.78kg) were used. Lambs were blocked according to live weight and randomly allocated to one of 6 dietary treatments. The experiment consisted of two 3 week periods, during which lambs were housed in individual slatted floor pens for a nine day period of adaptation to the experimental diets, and then transferred to metabolism crates for a further five day adaptation period and a seven day collection period. Lambs were kept under continuous lighting with free access to water at all times.

5.2.2. Diets and feeding procedure

The diet formulation and feeding pattern were as described in Chapter 4, with the exception that all lambs received 1.0 kg fresh weight per day offered in two equal meals at 09.00h and 16.00h throughout the trial. This level of feeding was equivalent to feeding a 28kg lamb for 175g daily live weight gain according to AFRC (1993) recommendations.

5.2.3. Data collection

5.2.3.1. Feed.

Samples of each diet were taken fortnightly throughout the trial and bulked prior to analysis.

5.2.3.2. Urine and faeces

During the seven day collection period, a total urine and faecal collection was carried out. Total urine output was collected daily into buckets containing 100ml of 1M H_2SO_4 to ensure a final pH of <3. Urine was filtered through glass wool, weighed and water was added to a final weight of 4.0kg. The diluted urine was mixed thoroughly and a subsample (approximately 60ml) taken and stored at –20°C prior to analysis. Total faecal output was collected daily using faecal collection harnesses, weighed and a sub sample (10%) taken and stored at –20°C for subsequent analysis.

5.2.4. Chemical Analysis

5.2.4.1. Feed analysis

Feed samples were analysed for dry matter (DM), organic matter (OM), nitrogen (N) and neutral detergent fibre (NDF) according to the methods described in Chapter 2.

5.2.4.2. Urine and faeces.

Urine samples were thawed and bulked proportionally to daily output for each sheep prior to analysis for N and purine derivatives as described in Chapter 2. Faecal samples were bulked proportionally to daily output for each sheep and analysed for DM, N, OM and NDF as described in Chapter 2.

5.2.5. Calculations and Statistical Analysis

Digestibility of DM, OM, N and NDF were calculated as;

$$\text{Apparent digestibility} = \frac{\text{Intake (g)} - \text{Excretion (g)}}{\text{Intake (g)}} \qquad \text{Equation 5.1}$$

Microbial N flow was calculated according to the method of Chen *et al.* (1992) as described in Chapter 2.

Results were analysed using analysis of variance for a two way randomised block design as described in Chapter 4. Additionally results for each energy source were analysed individually using analysis of variance for a one way randomised block design as described in Chapter 4.

5.3. RESULTS

5.3.1. Feed analysis and nutrient intakes.

The proximate analysis of the experimental diets is shown in Table 5.1. The organic matter contents of the synchronous diets (BS and SS) were similar to those predicted (Table 4.1), however the CP content of both diets were slightly higher than predicted. Supplying the slowly degradable protein sources in the morning feed and the rapidly degradable protein sources in the evening feed (treatments BI and SI) resulted in similar amounts of crude protein being supplied in each feed, whilst supplying all the major protein components of the diet in the morning feed resulted in a crude protein supply of 247g and 250g in the morning, and 61g and 65g in the evening for treatments BA and SA respectively.

Table 5.1. Proximate analysis (g/kgDM) of diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (A) for their hourly release of nitrogen and organic matter in the rumen.

	BS	BI		BA	
		am	pm	am	pm
Dry Matter (g/kg)	872.6	856.5	855.3	836.2	869.9
Organic Matter	936.6	931.5	922.3	947.2	931.0
Crude Protein	153.9	144.8	165.5	247.3	60.7
NDF	441.4	357.5	523.0	317.7	563.5
	SS	SI		SA	
		am	pm	am	pm
Dry Matter (g/kg)	891.4	892.0	889.2	882.0	886.9
Organic Matter	899.9	895.9	878.6	908.5	899.7
Crude Protein	157.3	170.6	144.7	250.4	64.8
NDF	555.6	526.6	544.6	437.6	621.8

Nutrient intakes over the 7 day collection period are presented in Table 5.2. Lambs on the barley based diet had significantly lower intakes of dry matter (5.98kg *vs.* 6.22kg), N (0.15kg *vs.* 0.16kg) and NDF (2.66kg *vs.* 3.36kg) than lambs on the sugar beet based diet. Lambs on the synchronous treatments had significantly higher NDF intakes than lambs on the asynchronous treatments. This was primarily due to differences in the NDF content of the sugar beet diets, the overall values being 555.6 and 529.7 g/kgDM for treatments SS and SA respectively.

Table 5.2. Total nutrient intakes (kg) of diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (A) for their hourly release of nitrogen and organic matter in the rumen.

	BS	BI	BA	SS	SI	SA	s.e.d.	Significance		
								En	Sync	Inter
DM	6.11	5.93	5.91	6.25	6.23	6.19	0.091	***	NS	NS
OM	5.72	5.49	5.55	5.63	5.53	5.60	0.085	NS	NS	NS
N	0.15	0.15	0.15	0.16	0.16	0.16	0.002	***	NS	NS
NDF	2.70	2.66	2.61	3.47	3.34	3.28	0.040	***	***	NS

5.3.2. Diet digestibility

There were no significant differences between treatments in terms of N digestibility (Table 5.3). Lambs on the sugar beet pulp diets showed significantly higher OM and NDF digestibilities ($p<0.001$) than lambs on the barley diets with mean values of 0.63 and 0.67 for OM digestibility and 0.43 and 0.62 for NDF digestibility for lambs on barley based and sugar beet based diets respectively. Within energy source there were no significant effects of treatment on nutrient digestibility.

Table 5.3. Apparent nutrient digestibility in lambs offered diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (A) for their hourly release of nitrogen and organic matter in the rumen.

								Significance		
	BS	BI	BA	SS	SI	SA	s.e.d.	En	Syn	Inter
N	0.62	0.63	0.64	0.67	0.67	0.66	0.016	NS	NS	NS
OM	0.62	0.63	0.64	0.67	0.67	0.66	0.012	***	NS	NS
NDF	0.41	0.44	0.44	0.63	0.63	0.60	0.026	***	NS	NS

5.3.3. Microbial protein production

Lambs on the barley based diets had a significantly higher level of PD excretion (14.4 *vs.* 10.6mM/d) and microbial N production (12.4 *vs.* 9.1g/d) than lambs on the sugar beet diets ($p<0.05$) (Table 5.4.). Within the barley based diets lambs on diet BS had a higher PD excretion and microbial N flow than lambs on either diet BI or BA but the difference did not reach statistical significance ($p=0.2$).

Table 5.4. Microbial N production in lambs offered diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (A) for their hourly release of nitrogen and organic matter in the rumen.

								Significance		
	BS	BI	BA	SS	SI	SA	s.e.d.	En	Syn	Inter
PD excretion (mM/d)	16.4	13.1	13.7	10.8	11.5	9.6	1.57	***	NS	NS
Mic N flow (g/d)	14.2	11.3	11.8	9.2	9.8	8.2	1.39	***	NS	NS

5.3.4. NITROGEN BALANCE

There were significant differences between energy sources in terms of N intake per day (Table 5.5). Lambs on barley based diets had a lower N intake than lambs on the sugar beet based diets (21.75g *vs.* 22.4g. $p<0.001$). There were no significant differences in faecal N output or urinary N output due to either energy source or synchrony.

In terms of the quantity of N retained on a daily basis, lambs on the barley based diets retained significantly less than lambs on the sugar beet diets (5.73g/d and 7.08g/d respectively. $p<0.05$). There was no significant difference due to synchrony, but there was a significant interaction between energy source and synchrony. On the barley based diets lambs on the asynchronous treatment retained the least N per day, and on the sugar beet base diets lambs on the asynchronous treatment retained the most N per day. When the two energy sources were analysed individually the lambs on diet BA showed significantly lower N retention on a daily basis than lambs on diets BI or BS ($p<0.05$).

In terms of N retention relative to N intake, lambs on the barley based diets retained a lower proportion of N intake than lambs on the sugar beet based diets (0.26 *vs.* 0.32. $p<0.05$). There were no significant effects of synchrony, but there was a significant interaction between energy source and synchrony. On barley based diets, lambs on the asynchronous treatment retained the lowest proportion of N intake, whilst on the sugar beet diets lambs on the asynchronous treatment retained the highest proportion of N intake. When the two energy sources were analysed individually there was a tendency for lambs on treatment BA to retain less N on a daily basis than lambs on treatments BI or BS ($p<0.1$).

Table 5.5. Nitrogen balance (g/d) of lambs offered diets based on either barley (B) or sugar beet pulp (S) and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (A) for their hourly release of nitrogen and organic matter in the rumen.

								Significance		
	BS	BI	BA	SS	SI	SA	s.e.d.	En	Syn	Inter
N intake (g/d)	21.5	21.0	20.8	22.5	22.5	22.3	0.32	***	NS	NS
Faecal N output (g/d)	7.10	6.43	6.69	7.02	6.68	6.99	0.374	NS	NS	NS
Urinary N output (g/d)	8.66	8.75	10.29	8.78	9.17	7.42	1.033	NS	NS	NS
N retained (g/d)	5.73	5.82	3.84	6.68	6.61	7.95	0.942	**	NS	*
N retained (g/g intake)	0.27	0.28	0.18	0.30	0.29	0.36	0.044	*	NS	*

5.4. DISCUSSION

5.4.1. TOTAL NUTRIENT INTAKE

As all lambs were offered the same fresh weight of feed daily the difference in DM intake between barley and sugar beet based diets may be attributable to the lower dry matter content of the barley based diet compared to the sugar beet based diet. This, combined with the lower N and NDF content of the barley based diets, lead to the significantly lower intake of N and NDF by lambs on these treatments. However , the barley based diets had a higher content of OM than the sugar beet based diets resulting in no difference in total OM intake. The difference in NDF intake between the synchronous and asynchronous diets may be attributable to the lower NDF content of diet SA compared to diet SS. Also, due to the fact that all lambs were offered the same amount of feed daily, the variation in nutrient intake within treatment was very small, therefore statistically significant differences between treatments were found when the numerical differences were small.

5.4.2. DIET DIGESTIBILITY

Whole tract diet digestibility is an important aspect of nutrition as it determines the proportion of consumed nutrients which ultimately becomes available to the animal for absorption and metabolism (Mertens, 1993). The digestibility of a particular diet is affected by both feed and animal factors. Feed factors include the solubility of the various components of the diet, which is determined by the chemical composition of each feed ingredient (e.g. starch *vs.* structural carbohydrate) and the type and degree of processing (Mertens, 1993). Animal factors include stage of production and level of intake (Mertens,

1993). In the current experiment, all animals were at the same stage of production and level of intake, and all feed was processed and mixed in the same manner, so any differences seen should have been due to the chemical composition of the ingredients used. The higher OM and NDF digestibility seen in lambs fed the sugar beet based diets may be partly attributed to the high pectin content of sugar beet pulp. Although classified as a structural carbohydrate, pectin is rapidly soluble (van Soest, 1982; Marouneck *et al.*, 1985) and occurs in much higher proportions in dicotyledon cell walls than monocotyledon (Chesson and Forsberg, 1997). The NDF fraction within barley grain arises principally from the seed coat, which is known to be highly indigestible (Campling, 1991) due to the high cellulose content (Chesson and Forsberg, 1997). Indeed, Marouneck *et al.* (1985) reported the dry matter digestibility of sugar beet pulp to be higher even than that of maize or sorghum, both feedstuffs containing high levels of starch. Ben-Ghedalia *et al.* (1989) reported that although overall OM digestibility was similar on both pectin rich and starch rich diets, cell wall digestibility was 16% units greater on the pectin rich diets. In the current experiment the mean cell wall (NDF) digestibility was 43% and 62% on the barley based and sugar beet based diets respectively, a difference of 19% units. NDF digestibility may also be affected by energy source through the effects on rumen pH. Starch based diets lead to a more rapid fermentation, resulting in a lowering of rumen pH, and an inhibition of fibre digestion (Mould and Ørskov, 1983).

The higher body fat deposition seen in the barley fed lambs in the previous experiment (Chapter 4) would suggest that OM digestibility, and therefore the supply of energy yielding substrates to the rumen, would be greater on the barley based diets. However the results from the current experiment would suggest the opposite. Therefore the differences in energy metabolism seen in the experiment reported in Chapter 4 between lambs on the barley and sugar beet based diets may be due to differences in some aspect of energy

utilisation rather than differences in energy supply. The lack of effect of synchrony on diet digestibility would suggest that the differences in energy metabolism due to synchrony seen in the previous experiment may also be due to differences in energy utilisation rather than to differences in the supply of energy yielding substrates from the diet.

5.4.3. MICROBIAL PROTEIN PRODUCTION

Inclusion of a readily fermentable energy source in ruminant diets has previously been shown to increase the efficiency of microbial protein production (Stern *et al.*, 1978; Herrera-Saldana *et al.*, 1990; Henning *et al.*, 1991; Moorby *et al.*, 1996). Although there is general agreement that energy source is the primary influence on microbial protein production (Henning *et al.*, 1991, 1993; Shabi *et al.*, 1998) there is some disagreement as to whether synchronising energy and protein supply to the rumen improves microbial protein production. Henning *et al.* (1991, 1993) found no effect of synchrony on microbial growth either *in vitro* or *in vivo*. However other workers have found microbial growth rates or efficiency to be significantly improved by dietary synchrony (Matras *et al.*, 1991; Sinclair *et al.*, 1993, 1995).

The mean daily production of microbial N in the current experiment (10.7g/d) was higher than reported by Witt *et al.* (1999b) who reported a value of 6.0 g/d, but similar to those reported by Henning *et al.* (1993) and Sinclair *et al.* (1993, 1995) in sheep fed at a similar level of intake. The results from the current experiment would seem to suggest that energy source had the greatest influence on microbial protein production, with lambs on the diet containing a rapidly degradable energy source (barley) having a significantly higher level of PD excretion and microbial N flow than lambs on the more slowly degradable energy

source (sugar beet pulp). Within energy source however, the degree of sychronisation between energy and N supply to the rumen, did not have a significant effect. However there was a tendency for lambs on the synchronous barley treatment to excrete a greater quantity of PD than lambs on the other two barley treatments, a finding which supports those of Sinclair *et al.* (1993, 1995). Within the sugar beet diets however the tendency for lambs on the intermediate diet to excrete a greater quantity of PD than lambs on the other two sugar beet treatments is more difficult to explain.

Overall the results from the current experiment are in agreement with those of Henning *et al.* (1993) who concluded that ruminal energy supply was of primary importance in determining microbial N production, but that some secondary advantage may be gained in synchronising the pattern of N supply.

5.4.4. NITROGEN BALANCE

In light of the fact that microbial N supply appears to have been higher in the lambs fed barley based diets than in the lambs fed sugar beet based diets, and total N intake lower, it might be expected that N retention may have been higher, due to a more efficient capture of rumen ammonia by the microbes. However the quantity of N excreted in the urine of barley fed lambs was greater than that of the sugar beet fed lambs (although the difference was not statistically significant), indicating that greater amounts of ammonia may have been absorbed from the rumen and subsequently excreted. This effect may be attributed to the higher inclusion of urea in the barley based diets leading to a rapid release of ammonia in the rumen post feeding, and may have contributed to the lower calculated N retention. The results from the corresponding growth trial reported in Chapter 4 would seem to

support this hypothesis, as lambs on the barley based diets showed significantly greater post feeding peaks in rumen ammonia concentration, and significantly higher plasma ammonia concentrations than the lambs on the sugar beet based diets.

The sugar beet based diets contained a higher proportion of slowly degraded protein sources (rape seed meal) and it is possible that these diets resulted in a greater post ruminal supply of undigested feed protein. This may have resulted in a different pattern of amino acids being available for absorption in the lambs fed sugar beet based diets.

Within the barley based diets the higher urinary N excretion in lambs fed diet BA compared to those fed diets BI or BS indicates a less efficient capture of ammonia N by the rumen microbes. The lower urinary N output in lambs on diet SA compared to those on diet SI and SS is more difficult to explain, as it implies that capture of ammonia N by the rumen microbes was improved by feeding an asynchronous diet. This is in agreement with the results of Henderson *et al.* (1998) who reported increased efficiency of microbial protein synthesis and increased microbial protein flow to the duodenum in lactating dairy cows fed asynchronous diets.

Witt *et al.* (1999b) reported no significant differences in the proportion of dietary N retained in lambs offered synchronous or asynchronous diets based on either rapidly or slowly degradable energy sources. The quantity of N retained (g/d) in the current experiment was similar to that reported by Witt *et al.* (1999b) for lambs of a similar live weight fed similar diets, but was generally higher than that reported by Henning *et al.* (1993), possibly due to the sheep used by Henning *et al.* (1993) having a mean live weight of 56kg, compared with 28 kg in the current experiment. As the lambs used by Henning *et al.* (1993) were more mature than those used in the current experiment they would

therefore have a lower proportion of crude protein in the live weight gain than those in the current experiment (Friggens *et al.*, 1997; Zygoyiannis *et al.* 1997).

The nitrogen balance technique is commonly used to investigate short-term changes in N retention in response to changed dietary regimens (MacRae *et al.*, 1993). When using this technique the assumption is made that it reflects whole body N accretion, however there are a number of studies which now question this assumption (Manantt and Garcia, 1992; MacRae *et al.*, 1993). MacRae *et al.* (1993) reported an overestimation of approximately 24% when the results from an N balance experiment were compared to those from a comparative slaughter study. In the current experiment the estimated N retention (g/d) was 44% greater than that determined in the experiment described in Chapter 4. One major source of error in N balance trials is the underestimation of N output (MacRae *et al.*, 1993; Spanghero and Kowalski, 1997). Underestimation of N loss in faeces and urine may be due to incomplete collection of material, or to the loss of volatile N compounds during collection of urine, or during drying of faecal samples (Spanghero and Kowalski, 1997). Spanghero and Kowalski (1997) reported that drying of faecal samples decreased the N concentration within the sample by around 15%, which would lead to significant underestimation of faecal N excretion. Another possible source of error may be the loss of volatile N compounds from the urine. In the current work urine was acidified on collection in order to minimise these losses. A summary of results from a number of N-balance experiments using sheep of similar live weight (mean live weights range from 27kg (s.d. 0.9kg) to 29kg (s.d. 5.5kg)) is presented in Table 5.6. Each of the figures presented in Table 5.6 is the mean of several treatments, which include a wide range of dietary regimens, so direct comparisons are difficult to make. However it is interesting to note that the work in which faecal N was determined in fresh rather than dried samples (Wiegand *et al.*, 1995), produced the lowest calculated N balance.

Table 5.6. Summarised nitrogen balance data from published trials using sheep. (g/d).

N Intake	Faecal N	Urine N	N Balance	Reference
10.01	5.42	0.33	4.25	Chriyaa *et al.*, 1997
14.72	6.21	4.48	5.55	Bonsi and Osuji. 1997
15.2	7.7	4.1	3.3	Weigand *et al.*, 1995
18.38	5.68	6.85	5.83	Witt *et al.*, 1999b
31.22	8.99	13.83	8.29	Seal *et al.*, 1993
21.77	6.83	8.85	6.11	Current work

5.5.CONCLUSIONS.

The results from the current experiment indicate that energy source within the diet significantly affects OM and NDF digestibility, but the degree of synchrony does not. Therefore the differences in energy metabolism seen in previous experiments cannot be attributed to differences in energy digestibility. Energy source also had a significant effect on microbial N flow to the duodenum and N retention, with the rapidly degradable energy source (barley) producing the greatest flow of microbial N, but the slowly degradable energy source (sugar beet pulp) resulting in greater N retention. Although there are indications that rumen synchrony may have an effect on microbial N flow, the differences were not significant.

CHAPTER 6.

THE EFFECTS OF SEQUENCE OF CONCENTRATE ALLOCATION ON THE METABOLISM AND PERFORMANCE OF GROWING LAMBS FED A PORTION OF THE DIET *AD LIBITUM*.

6.1. INTRODUCTION.

In the growth trial described in Chapter 4, it was found that restricted fed lambs on a barley based diet deposited significantly less fat in the carcass when the diet was formulated to be asynchronous in terms of energy and nitrogen release in the rumen, indicating an alteration in energy metabolism. Similar results have been reported in both restricted and *ad libitum* fed sheep (Witt *et al.*, 1999a, b). However in both the experiments reported by Witt *et al.* (1999a, b) the diets compared were formulated from different ingredients, and it has been suggested that the effects seen may have been due to an influence of the feed ingredients rather than to synchrony. The aim of this trial was to investigate whether the effects seen in the barley fed lambs in the experiment described in Chapter 4 would also be seen in lambs offered a portion of the diet *ad libitum*.

6.2. MATERIALS AND METHODS.

6.2.1. Experimental Animals

Thirty two entire male Charrolais cross lambs (Charrolais x (Friesland x Lleyn) or Charrolais x (Charrolais x Lleyn)), with an initial live weight of 25.8kg (s.d. 2.31 kg) were used. Lambs were blocked according to breed and live weight, and then randomly allocated to one of three dietary treatments or to an initial slaughter group. Lambs were housed in individual slatted floor pens under continuous lighting with free access to water. Lambs were penned 12 days before the start of the trial, allowing 7 days adaptation to the pen while remaining on *ad libitum* creep feed (Lambmaster, Wynnstay Farmers. 16% crude protein, 4% oil, 8% fibre, 6% ash), and five days for adaptation to the experimental diets. The experimental diet was introduced at a rate of 20% dry matter intake per day.

6.2.2. Diets and feeding procedure

Using the previously determined degradability constants and computer program described in Chapter 3, a diet was formulated to have an approximate ME content of 10.5 MJ/kgDM and a CP content of 150g/kgDM. The diet used in the current experiment was based on the barley diet used in the experiment described in Chapter 4 and was manufactured using the same batches of ingredients used in the previous 3 experiments. In the current trial however the diet was split into a basal component and two protein components as described in Table 6.1. Different degrees of synchrony were achieved by altering the sequence of allocation of the various components of the diets. The basal portion of the diet supplied the main energy and fibre sources (barley and wheat straw), and was fed *ad libitum*. Protein A provided the major slowly degradable N sources (rape seed meal and distillers), and protein B the major rapidly degradable N sources (distillers and urea). The allocation of each of the feeds is presented in Table 6.2. Lambs on the synchronous treatment (S) received equal quantities of rapidly and slowly degradable protein

components (Proteins A and B) in both the morning and evening feed, whilst lambs on the intermediate treatment (I) received the majority of the slowly degradable protein sources (Protein A) in the morning feed and the majority of the rapidly degradable protein sources (Protein B) in the evening feed, and lambs on the asynchronous treatment (A) received all of the major protein components of the diet in the morning feed.

Table 6.1. Diet formulation and predicted composition (g/kgDM)

Ingredient	Basal	Protein A	Protein B
Wheat Straw	419	0	0
Barley	550	0	0
Distillers grains	0	684	935
Rape seed meal	0	316	0
Urea	0	0	65
Vitamins and minerals*	31	0	0
Organic matter	929.9	934.3	954.3
Crude protein	64.1	287.4	425.3
Metabolisable energy (MJ/kgDM)	9.9	12.8	12.2
Fermentable ME (MJ/kgDM)	9.4	11.0	9.8
Effective rumen degradable protein	36.1	192.4	173.2
Digestible undegradable protein supply	34.4	22.5	0
Metabolisable protein supply	93.8	92.1	62.2

** Trouw Intensive Lamb, Trouw UK, Northwich, Cheshire. This provided on a total diet basis (g/kg) Ca, 1.6; P, 0.58; Na, 6.0: vitamins (mg/kg): retinol, 2.37; cholecalciferol, 0.033; α-tocopherol, 13: trace minerals (mg/kg): Fe, 26; Co, 1.3; Mn, 26; Zn, 33; I, 2; Se, 0.2.

Refusals were weighed back twice weekly and the basal diet was offered at 120% of the previous week's intake. The protein components of the diet were fed in two meals at 09.00 and 16.00h at a level sufficient for a live weight gain of 175g/d according to AFRC (1993) recommendations, and the quantity of protein fed was recalculated weekly according to the live weight recorded the previous day. For the purposes of this trial it was necessary that

all the protein feed was consumed within one hour of feeding, and to achieve this the basal diet was removed for one hour prior to feeding the protein meals (i.e. 08.00h – 09.00h and 15.00h – 16.00h).

Table 6.2. Pattern of allocation of protein feeds (proportion of total daily allowance).

	S		I		A	
	am	pm	am	pm	am	pm
Protein A	0.5	0.5	1.0	0	1.0	0
Protein B	0.5	0.5	0	1.0	1.0	0

6.2.3. Data collection.

6.2.3.1. Feed

Samples of each feed were taken fortnightly throughout the trial and bulked prior to analysis.

6.2.3.2. Live weight and carcass characteristics

Lambs in the initial slaughter group were killed on day 0 of the experiment and processed as described in Chapter 4. Remaining lambs were weighed at 14.00h on the same day each week and on reaching 40kg were sheared prior to slaughter. All lambs and carcasses were processed as described in section 4.2.

6.2.3.3. Blood metabolites

At 34.2kg (s.d. 2.81kg) live weight six lambs from each treatment were blood sampled at intervals during the day as described in section 4.2, and the plasma analysed for ammonia, urea and β-hydroxybutyrate as described in section 2.5.

6.2.3.4. Rumen metabolites.

On reaching 36.6kg (s.d. 2.06kg) live weight 4 lambs from each treatment were rumen sampled at intervals during the day as described in Chapter 4, and the rumen fluid analysed for pH, ammonia and volatile fatty acids as described in Chapter 2.

6.2.3.5. Intake monitoring

In order to determine the pattern of intake of the basal diet over a 24 hour period the feed boxes for six lambs from each treatment were placed on balances connected to a Squirrel data logger (Grant 1250 series) which recorded the weight of the feed box at five minute intervals. The pattern of intake was determined by dividing the 24 hour period into six 4 hour periods and calculating the proportion of total DMI consumed during each period.

6.2.4. Chemical analysis

6.2.4.1. Feed

Feed samples underwent a full analysis according to the methods described in Chapter 2.

6.2.4.2. Carcass and non carcass components

The frozen half carcasses and non carcass components were ground separately, once through a 13mm screen and twice through a 4mm screen in a whole carcass grinder (Wolfking; Model c160-uni). The ground samples were mixed thoroughly and sub-samples taken (approx. 1kg). Samples were analysed for DM, OM, CP and EE as described in Chapter 2. Gross energy content of the whole body was determined by calculation, assuming that CP and EE had energy values of 23.6 and 39.9MJ/kg respectively (ARC, 1980).

6.2.5. Statistical analysis.

Results were analysed by analysis of variance using a one-way, randomised block design. The blood and rumen data were also analysed using repeated measures ANOVA with degree of synchrony (S, I, A) forming the "between animal" stratum and sample time and associated interactions with synchrony forming the "within animal by time" stratum. All statistical analysis was performed using Genstat for Windows (Version 5, Release 3.22), and a significant difference was declared at $p<0.05$. The skeleton ANOVAs are presented below.

Table 6.3. One way ANOVA used for statistical analysis of all growth, intake and carcass measurements.

Source	d.f.
Blocks	7
Synchrony	2
Residual	14
Total	23

Table 6.4. One way ANOVA used for statistical analysis of all blood metabolites.

Source	d.f.
Blocks	5
Synchrony	2
Residual	10
Total	17

Table 6.5. One way ANOVA used for statistical analysis of all rumen metabolites.

Source	d.f.
Blocks	3
Synchrony	2
Residual	6
Total	11

Table 6.6. Repeated measures ANOVA used for statistical analysis of all blood metabolites.

Source	d.f.
Block	5
Treatment	2
Residual	10
Time	6
Time.Treatment	12
Residual	90
Total	125

Table 6.7. Repeated measures ANOVA used for statistical analysis of all rumen metabolites.

Source	d.f.
Block	5
Treatment	2
Residual	10
Time	5
Time.Treatment	10
Residual	75
Total	107

6.3. RESULTS

6.3.1. Feed analysis and nutrient intakes.

The proximate analysis of each feed is shown in Table 6.8.

Table 6.8. Proximate analysis of experimental diets

Component (g/kgDM)	Basal	Protein A	Protein B
Dry matter (g/kg)	860.1	867.1	842.6
Organic matter	934.7	944.8	953.7
Crude protein	65.8	301.2	405.7
NDF	432.7	345.7	355.9
Ether extract	5.0	36.9	24.3
GE (MJ/kgDM)	15.3	17.5	17.1

The organic matter and crude protein contents of all three feeds were similar to the predicted values. The basal diet had the lowest contents of organic matter, ether extract and crude protein, and had the highest ash content. Protein A had the highest ether extract content, whilst Protein B had the highest crude protein content.

Table 6.9. Total nutrient intakes over the experimental period of lambs fed barley based diets formulated to be synchronous (S), intermediate (I) or asynchronous (A) in terms of hourly nitrogen and organic matter release in the rumen.

	S	I	A	s.e.d.	Significance
Basal (kg DM)	61.6	62.4	65.1	3.26	NS
Protein A (kg DM)	8.3	8.9	8.2	0.24	*
Protein B (kg DM)	5.1	5.5	5.0	0.15	*
Dry matter (kg)	75.0	76.7	78.3	3.39	NS
Organic matter (kg)	70.3	71.9	73.4	3.17	NS
Crude protein (kg)	8.64	8.99	8.80	0.283	NS
Neutral detergent fibre (kg)	31.4	32.0	32.8	1.46	NS
Ether extract (kg)	0.737	0.769	0.747	0.023	NS
Gross energy (MJ)	1176	1203	1226	52.2	NS

* = $p<0.05$, ** = $p<0.005$, *** = $p<0.001$, NS = $p>0.05$.

All protein feeds were consumed within one hour of feeding, and there were no refusals. There were no significant differences between treatments in total intake of basal diet (Table 6.9). However lambs on treatment I appeared to have a significantly higher intake of both protein diets than lambs on either of the other two treatments ($p<0.05$), but this did not translate into a significant difference in total CP intake.

6.3.2. Pattern of intake

The proportion of total daily dry matter intake of the basal diet in each of six four hour periods is presented in Table 6.10.

Table 6.10. Proportion of total daily dry matter intake of basal diet in *each of six four hour periods in lambs fed barley based diets formulated to be synchronous (S), intermediate (I) or asynchronous (A) in terms of hourly nitrogen and organic matter release in the rumen.*

	S	I	A	s.e.d.	Significance
09:00 – 13:00	0.423	0.347	0.382	0.0709	NS
13:00 – 17:00	0.031	0.085	0.056	0.0214	NS
17:00 – 21:00	0.414	0.365	0.398	0.0668	NS
21:00 – 01:00	0.054	0.103	0.074	0.0276	NS
01:00 – 05:00	0.039	0.029	0.028	0.0239	NS
05:00 – 09:00	0.038	0.070	0.063	0.0296	NS

* = $p<0.05$, ** = $p<0.005$, *** = $p<0.001$, NS = $p>0.05$.

There were no significant differences between treatments in the proportion of the total daily intake of basal diet consumed during any one period. However there was a significant effect of time, with high proportions of total intake occurring during the periods immediately after fresh basal diet was offered.

6.3.3. Growth rate and feed conversion efficiency.

There were no significant differences between treatments in daily live weight gain or feed conversion efficiency (FCE) (Table 6.11). Lambs on the intermediate treatment tended to be on the trial longer (approximately 4 days) but this was not statistically significant.

Table 6.11. Growth rate and feed conversion efficiency (FCE) of lambs on barley based diets formulated to be synchronous (S), intermediate (I) or asynchronous (A) in terms of hourly nitrogen and organic matter release in the rumen.

	S	I	A	s.e.d.	Significance
Initial live weight (kg)	26.8	27.0	26.6	0.94	NS
Slaughter weight (kg)	41.2	40.9	40.8	0.78	NS
Days on trial	44.4	47.9	44.4	2.02	NS
Live weight gain (kg/d)	0.336	0.293	0.315	0.0193	NS
Intake (gDM/d)	1712	1616	1786	87.7	NS
FCE (kg gain /kg DMI)	0.193	0.181	0.182	0.0111	NS

* = $p<0.05$, ** = $p<0.005$, *** = $p<0.001$, NS = $p>0.05$.

There were no significant differences between treatments in any of the carcass or non carcass characteristics measured (Table 6.12).

Table 6.12. Carcass measurements (kg) of lambs fed barley based diets formulated to be synchronous (S), intermediate (I) or asynchronous (A) in terms of hourly nitrogen and organic matter release in the rumen.

	S	I	A	s.e.d.	Significance
Cold carcass	17.9	18.0	18.1	0.33	NS
Killing out %	43.6	44.2	44.4	0.90	NS
Liver	0.854	0.789	0.827	0.032	NS
Kidney	0.109	0.109	0.109	0.0037	NS
Kidney fat	0.230	0.232	0.221	0.041	NS
Heart	0.167	0.168	0.177	0.0117	NS
Wool	0.931	0.910	0.872	0.0729	NS
Empty GIT	3.6	3.4	3.4	0.15	NS
Gut fill	5.7	5.9	5.7	0.29	NS
Total non-carcass components	13.7	13.3	13.4	0.49	NS

* = $p<0.05$, ** = $p<0.005$, *** = $p<0.001$, NS = $p>0.05$.

Chemical analysis of carcass and non-carcass components showed no significant differences between treatments in either composition or total quantity of DM, OM, CP or EE (Tables 6.13 and 6.14).

Table 6.13. Carcass analysis of lambs fed barley based diets formulated to be synchronous (S), intermediate (I) or asynchronous (A) in terms of hourly nitrogen and organic matter release in the rumen.

	S	I	A	s.e.d.	Significance
Water (g/kg)	596.5	600.0	609.1	10.30	NS
Ash (g/kg)	38.9	39.1	38.8	2.74	NS
CP (g/kg)	168.4	169.2	169.5	3.25	NS
EE (g/kg)	189.8	184.4	179.4	15.83	NS
Total DM (kg)	7.25	7.22	7.10	0.275	NS
Total OM (kg)	6.55	6.51	6.40	0.280	NS
Total CP (kg)	3.02	3.05	3.07	0.058	NS
Total EE (kg)	3.42	3.33	3.27	0.298	NS

* = p<0.05, ** = p<0.005, *** = p<0.001, NS = p>0.05.

Table 6.14. Non carcass analysis of lambs fed barley based diets formulated to be synchronous (S), intermediate (I) or asynchronous (A) in terms of hourly nitrogen and organic matter release in the rumen.

	S	I	A	s.e.d.	Significance
Water (g/kg)	686.6	677.6	679.1	11.72	NS
Ash (g/kg)	18.5	17.7	19.1	1.06	NS
CP (g/kg)	152.1	151.3	155.6	4.19	NS
EE (g/kg)	124.7	133.1	124.3	11.40	NS
Total DM (kg)	4.31	4.29	4.30	0.257	NS
Total OM (kg)	4.06	4.06	4.04	0.250	NS
Total CP (kg)	2.08	2.01	2.08	0.081	NS
Total EE (kg)	1.73	1.78	1.67	0.193	NS

* = p<0.05, ** = p<0.005, *** = p<0.001, NS = p>0.05.

There were no significant differences in the composition of the whole body (Table 6.15).

Table 6.15. Whole body composition of lambs on barley based diets formulated to be synchronous (S), intermediate (I) or asynchronous (A) in terms of hourly nitrogen and organic matter release in the rumen.

	S	I	A	s.e.d.	Significance
DM (g/kg)	11.56	11.51	11.4	0.45	NS
OM (g/kgDM)	10.61	10.57	10.44	0.46	NS
CP (g/kgDM)	5.09	5.06	5.14	0.0845	NS
EE (g/kgDM)	5.15	5.11	4.94	0.467	NS

* = p<0.05, ** = p<0.005, *** = p<0.001, NS = p>0.05.

There were no significant differences between treatments in nitrogen or energy balance (Tables 6.16 and 6.17).

Table 6.16. Nitrogen balance of lambs fed barley based diets formulated to be synchronous (S), intermediate (I) or asynchronous (A) in terms of hourly nitrogen and organic matter release in the rumen.

	S	I	A	s.e.d.	Significance
Initial N content (g)	597	597	597	0.0	NS
Final N content (g)	815	810	823	13.5	NS
N intake (kg)	1.38	1.44	1.41	0.047	NS
N deposited (g)	219	213	227	12.9	NS
N balance (g/d)	4.63	4.35	4.92	0.377	NS
N balance (g/g N intake)	0.158	0.147	0.161	0.0106	NS

* = p<0.05, ** = p<0.005, *** = p<0.001, NS = p>0.05.

Table 6.17. Energy balance of lambs fed barley based diets formulated to be synchronous (S), intermediate (I) or asynchronous (A) in terms of hourly nitrogen and organic matter release in the rumen.

	S	I	A	s.e.d.	Significance
Initial GE content (MJ)	179	179	179	0.0	NS
Final GE content (MJ)	323	320	316	18.51	NS
GE intake (MJ)	1176	1203	1226	52.2	NS
GE retained (MJ)	143	141	136	18.9	NS
Energy balance (MJ/d)	3.06	2.91	3.01	0.39	NS
Energy balance (MJ/MJ intake)	0.121	0.117	0.111	0.0127	NS

* = p<0.05, ** = p<0.005, *** = p<0.001, NS = p>0.05.

6.3.4. Rumen metabolites

6.3.4.1. Rumen pH

There were no significant differences between treatments in rumen pH at any time point (Figure 6.1.). However there was a significant effect of time, with lambs on all treatments exhibiting a cyclical pattern in relation to feeding. The highest rumen pH was seen at 08.00h (mean value of pH7.2), followed by a decline after the morning feeding to a mean value of pH6.4 at 12.30, a rise just before and immediately after the afternoon feeding, and a decline to the lowest mean value of pH6.3 at 19.30h.

6.3.4.2. Rumen ammonia

Daily rumen ammonia concentrations are shown in Figure 6.2. Overall there were no significant differences between treatments in rumen ammonia concentration. However there was a significant effect of time ($p<0.001$), and some significant differences between treatments at specific time points. At 08.00h there were no significant differences between treatments, the mean ammonia concentration being 24.1 mg/l. After this time point each treatment exhibited a different cyclical pattern of rumen ammonia, which followed the pattern of feeding of the nitrogen fraction of the diet. Lambs on the synchronous treatment showed two relatively small peaks of rumen ammonia concentration within 2 hours of feeding each protein meal (127mg/l at 10.30h and 87mg/l at 17.30h). Lambs on the intermediate diet exhibited a similar sized peak to the lambs on the synchronous diet at 10.30h (123mg/l), and a second, significantly larger peak of 242mg/l at 17.30h, whilst lambs on the asynchronous treatment exhibited one large peak at 10.30h (299mg/l) which was significantly greater than those on the other two treatments ($p<0.001$).

6.3.4.3. Rumen VFAs

Overall there were no significant differences between treatments in the total VFA concentration with mean values of 49.8, 62.1 and 57.1mM for lambs on synchronous, intermediate and asynchronous treatments respectively (Figure 6.3). There were also no significant differences between treatments in the molar proportions of VFAs with mean ratios of acetate:propionate:butyrate of 58:33:9, 52:34:14 and 56:37:8 for the synchronous, intermediate and asynchronous treatments respectively (Table 6.18).

Table 6.18. Molar proportions of rumen volatile fatty acids in *lambs fed diets based on barley and differing in sequence of allocation of individual ingredients to be synchronous (S), intermediate (I) or asynchronous (S) for their hourly release of nitrogen and organic matter in the rumen.*

	08.00	10.30	12.30	15.00	17.30	19.30
Acetate						
S	0.640	0.550	0.560	0.525	0.508	0.518
I	0.543	0.510	0.532	0.473	0.483	0.475
A	0.570	0.528	0.520	0.515	0.543	0.513
s.e.d.	0.0416	0.0289	0.0366	0.0151	0.0201	0.0215
Propionate						
S	0.262	0.275	0.320	0.350	0.338	0.360
I	0.267	0.318	0.348	0.335	0.365	0.330
A	0.305	0.343	0.363	0.368	0.348	0.375
s.e.d.	0.0612	0.0260	0.0509	0.0496	0.0295	0.0532
Butyrate						
S	0.058	0.120	0.078	0.078	0.108	0.083
I	0.138	0.130	0.083	0.150	0.115	0.163
A	0.068	0.088	0.078	0.075	0.070	0.073
s.e.d.	0.0456	0.0218	0.0118	0.0463	0.0266	0.0525

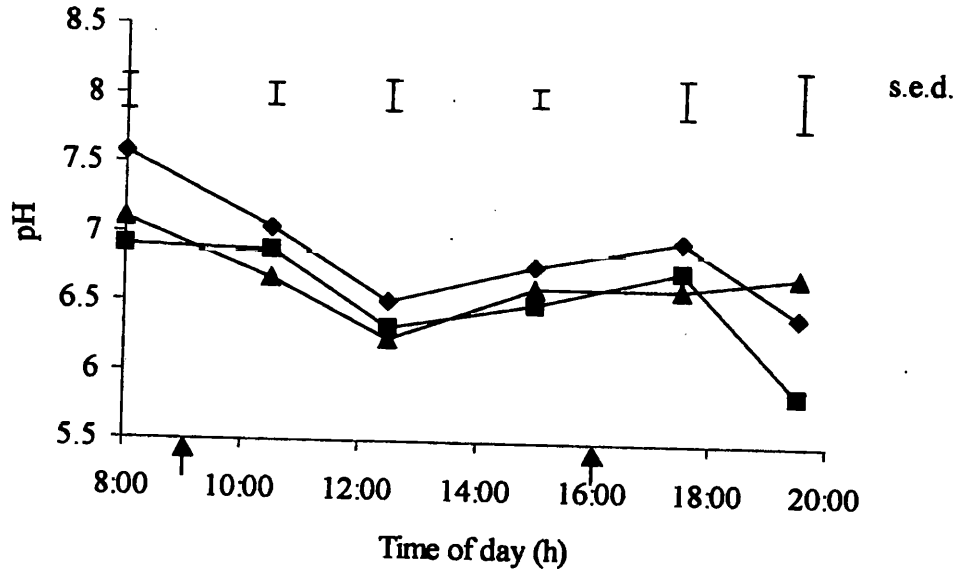


Figure 6.1. Rumen pH in lambs fed barley based diets formulated to be synchronous (◆), intermediate(■) or asynchronous(▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate times of feeding protein meals.

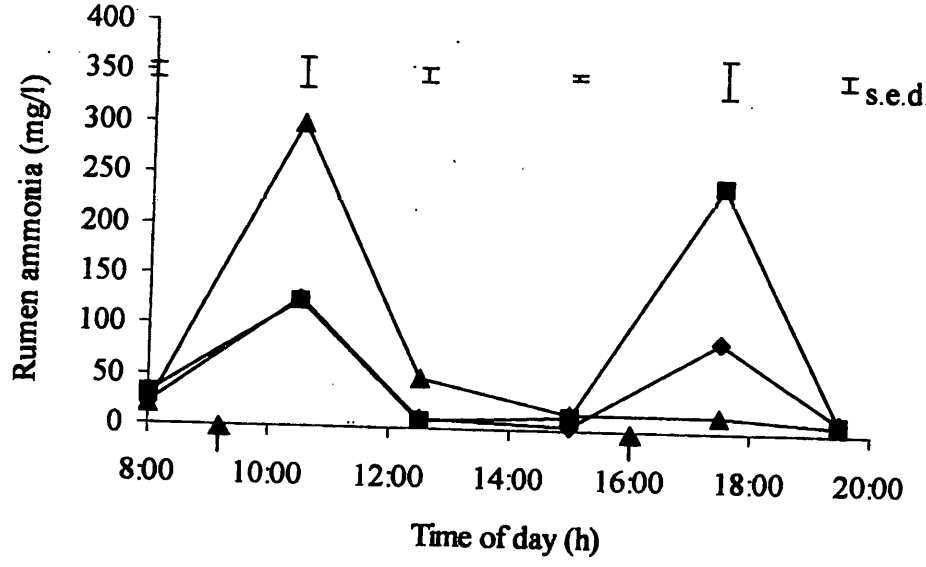


Figure 6.2. Rumen ammonia concentrations in lambs fed barley based diets formulated to be synchronous (◆), intermediate (■) or asynchronous (▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate times of feeding of protein meals.

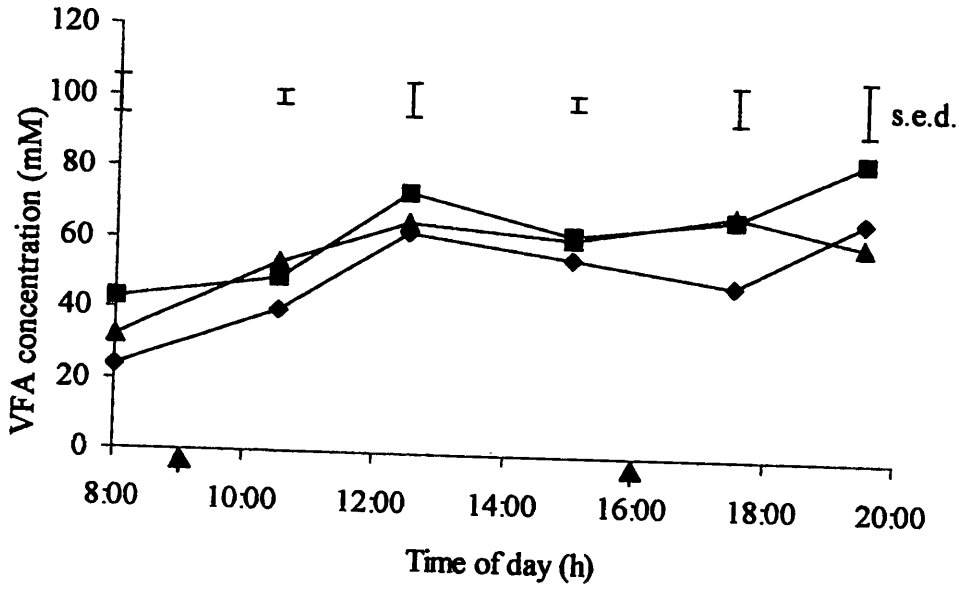


Figure 6.3. Total rumen VFA concentrations in lambs fed barley based diets formulated to be synchronous (♦), intermediate (■) or asynchronous (▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate times of feeding of protein meals.

6.3.5. Plasma Metabolites

6.3.5.1. Plasma ammonia

Overall there were no significant differences between treatments in plasma ammonia concentrations and values were generally low throughout the day (Figure 6.4), although there was a significant effect of time ($p<0.001$). All treatments exhibited a cyclical trend in plasma ammonia concentration throughout the day, with concentrations rising after each protein meal was consumed. A further significant rise was seen between 19.30h and 22.00h.

6.3.5.2. Plasma urea

Daily plasma urea concentrations are presented in Figure 6.5. There was a tendency for lambs on the asynchronous treatment to have the highest plasma urea concentrations overall ($p<0.1$), and there was a significant effect of time on plasma urea concentrations ($p<0.001$), and a significant time*synchrony interaction ($p<0.005$). Lambs on all treatments showed increases in plasma urea concentrations after consumption of the protein meals. There were no differences between treatments in plasma urea concentration at 08.00h, the mean concentration being 2.731mM. Lambs on the synchronous treatment showed a peak in plasma urea concentration at 10.30h (3.99mM) followed by a second smaller peak at 17.30h (2.99mM). Lambs on the intermediate treatment showed a decline in plasma urea concentration reaching a minimum value of 2.01mM at 12.30h, followed by a peak of 3.18mM at 19.30h, whilst lambs on the asynchronous treatment showed a single peak of 4.72mM at 10.30h, followed by a steady decline to a minimum value of 1.71mM at 22.00h.

6.3.5.3. Plasma β-hydroxybutyrate

The daily plasma β-hydroxybutyrate concentration is shown in Figure 6.6. There were no overall differences between treatments in plasma β-hydroxybutyrate concentration, and no differences were seen at any individual time points. However there was a significant effect of time, with all treatments showing postfeeding increases, with the maximum concentration of 0.44mM occuring at 19.30h.

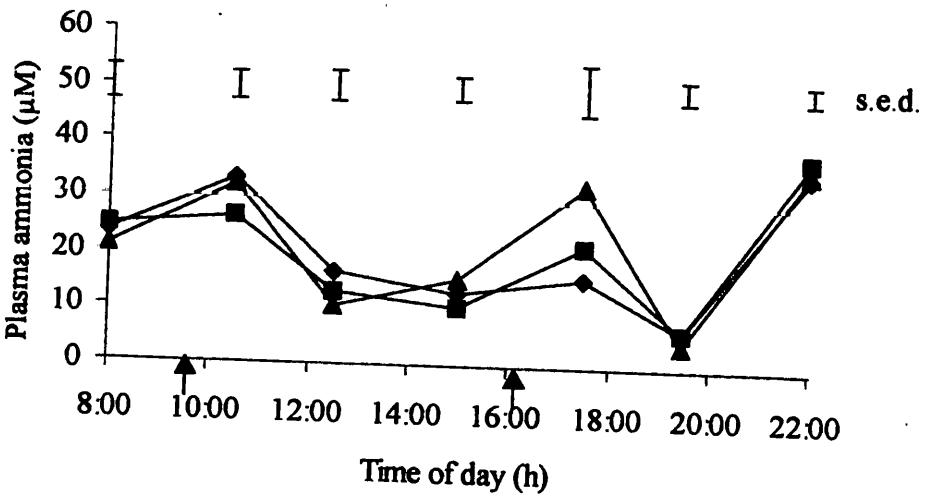


Figure 6.4. Plasma ammonia concentration in lambs fed barley based diets formulated to be synchronous (♦), intermediate (■) or asynchronous (▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate times of feeding of the protein meals.

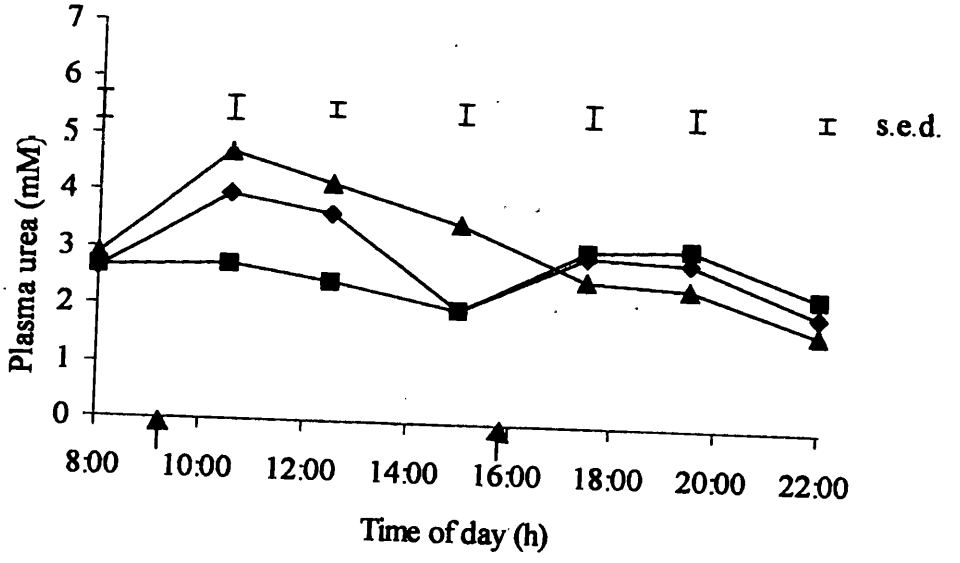


Figure 6.5. Plasma urea concentration in lambs fed barley based diets formulated to be synchronous (♦), intermediate (■) or asynchronous (▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate times of feeding of the protein meals.

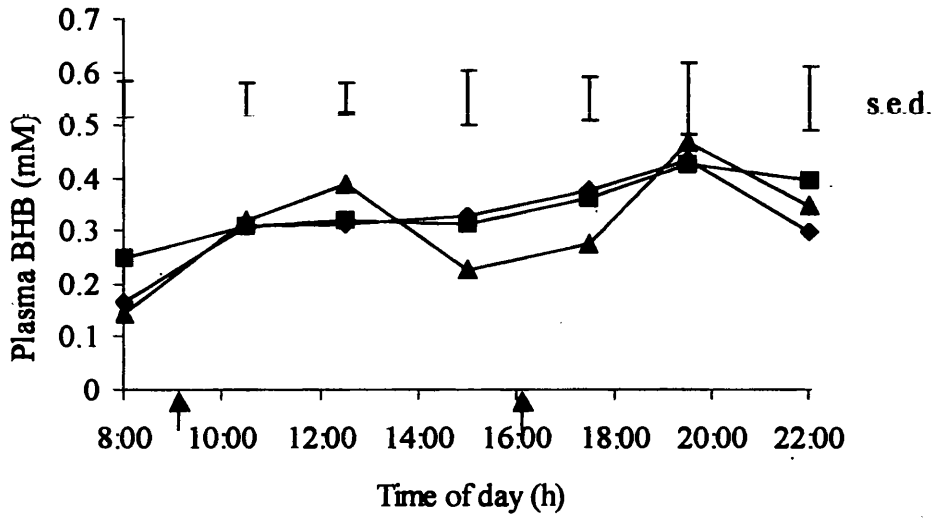


Figure 6.6. Plasma β hydroxybutyrate concentration in lambs fed barley based diets formulated to be synchronous (♦), intermediate (■) or asynchronous(▲) for their hourly release of nitrogen and organic matter in the rumen. Arrows indicate times of feeding of the protein meals.

6.4. DISCUSSION

6.4.1. Feed analysis and nutrient intakes.

In contrast to the results of the current experiment where dry matter intakes were similar across all treatments, Witt *et al.* (1999a) reported significantly lower dry matter intakes in *ad lib* fed lambs offered a synchronous diet containing a rapidly degradable energy source than in lambs offered an asynchronous diet containing a rapidly degradable energy source. However the diets used by Witt *et al.* (1999a) differed in the primary energy source, with the synchronous diet being based on barley, and the asynchronous diet being based on tapioca, so the difference in DMI may have been due to the difference in energy source. Although the total potential OM degradation is similar for both energy sources (0.9 *vs.* 0.87 for tapioca and barley respectively) the rate of degradation (c) is greater for barley (0.229) than for tapioca (0.098) (Witt *et al.*, 1999a). Ruminant animals fed concentrate diets where physical bulk is not a restriction on intake are capable of regulating their energy intake, probably through rumen receptors sensitive to VFAs, and liver receptors sensitive to substrate supply (Forbes, 1993). Therefore a more rapidly degradable energy source will cause a greater production of VFA in the rumen, and a greater availability of substrates to the liver, resulting in a reduction of feed intake.

Kyriazakis and Oldham (1997) proposed that sheep offered a choice of diets will tend to select those which promote a synchronous release of N and OM in the rumen. If this was the case then in the current experiment it might have been expected that those sheep receiving a large amount of rapidly degradable protein in a particular meal might have a higher intake of basal diet in the period following the protein meal. However there were no significant differences between treatments in intake of basal diet in any period of the day, although animals on all treatments did increase their intake of basal diet following

consumption of the protein meal. However fresh basal diet was offered after each protein meal, and therefore it is difficult to separate the effect the protein meal may have had on intake of basal diet from the stimulatory effect of offering fresh feed (Forbes *et al.*, 1995). The treatments used by Kyriazakis and Oldham (1997) investigated whether energy source affected the choice of nitrogen source by sheep. In the current trial nitrogen supply was altered and the effects on energy intake investigated. It may therefore be possible that energy source dictates choice of N supply but not *vice versa*.

High ruminal concentrations of ammonia have been shown to alter intake pattern (Conrad *et al.*, 1977), although not necessarily total daily intake (Köster *et al.*, 1997). The effects of ammonia on appetite are thought to be mediated by the excitatory amino acids L-glutamate and L-aspartate, which are key intermediates of ammonia metabolism in the brain (Cunningham *et al.*, 1994). These amino acids are also powerful appetite stimulants (Stanley *et al.*, 1993; Maldonadoirizarry *et al.*, 1995). It has also been shown that in rats a derivative of glutamate, γ- aminobutyric acid, is co-expressed with neuropeptide-Y, also a powerful appetite stimulant (Pu *et al.*, 1999). Therefore in situations where high plasma concentrations of ammonia occur, it is possible that the central nervous system becomes depleted of L-glutamate and L-aspartate, resulting in a depression of appetite. Although post feeding peaks in plasma ammonia concentration were seen in the current experiment, plasma ammonia concentrations did not rise above 38μM and no alteration on pattern of intake was apparent.

6.4.2. Growth and carcass composition.

Although protein was supplied at a level sufficient to support a growth rate of 175g/d, the mean growth rate in the current experiment was 315g/d. Figure 1.5 illustrates the fact that weight gain increases as protein supply increases, but that maximum response is

determined by availability of energy. MacRae and Lobley (1984) suggest that when energy is supplied in excess protein synthesis may increase, as glucogenic or ketogenic amino acids are not required as an energy source, and may therefore be utilised for protein synthesis. The maximum efficiency of protein utilisation has been shown to occur on diets which are protein limited (Gordon and McMurray, 1979; Satter and Roffler, 1975). Therefore it may be that in the current trial, as energy was supplied in excess, growth was not limited by energy availability, and the protein which was available was utilised with a high degree of efficiency.

The results of the current experiment in which there were no significant effects of synchrony on any aspect of growth or carcass composition are in contrast to those of Witt *et al.* (1999a) who reported improved feed conversion efficiencies in lambs fed synchronous diets compared to those fed asynchronous diets when offered *ad lib*. Witt *et al.* (1999a) also reported increased subcutaneous fat deposition in lambs fed a synchronous diet containing a rapidly degradable energy source (FS) compared to lambs fed an asynchronous diet containing a rapidly degradable energy source (FA), despite a lower DMI on diet FS, indicating that there may be some difference in energy metabolism. It has been suggested that synchronising energy and nitrogen supply to the rumen may increase ammonia assimilation by the rumen microbes, resulting in a decreased requirement for ammonia detoxification by the liver, and increasing the efficiency of microbial protein synthesis (Sinclair *et al.*, 1993, 1995). The increase in efficiency of microbial protein production reported by Sinclair *et al.* (1993, 1995) did not appear to result in an increase in the quantity of microbial protein being supplied post-ruminally, indicating a possible improvement in energy rather than protein metabolism in the rumen. Witt *et al* (1999a, b) also reported a possible improvement in energy rather than N metabolism in the whole animal, a result which appears to be confirmed by the results of the experiment described

in Chapter 4. Therefore in the current experiment where energy was supplied in excess it is perhaps not surprising that no differences in production were evident. Also the fact that at no time were the energy and protein components of the diet supplied together resulted in all the treatments being asynchronous. When calculated using measured hourly intake pattern of basal diet, the synchrony indices were 0.57, 0.57 and 0.6 for treatments S, I and A respectively.

The majority of experiments carried out to investigate the effect of synchrony on production in *ad lib* fed animals have used lactating animals fed forage based diets, and have generally concluded that synchrony does not have a major effect on milk production in either cows (Henderson *et al.*, 1998; Kolver *et al.*, 1998; Aldrich *et al.*, 1993) or ewes (Witt *et al.*, 2000).

6.4.3. Rumen metabolites.

6.4.3.1. Rumen pH.

The mean pH observed in the current experiment was pH6.7, close to the value of pH6.8 proposed by McCarthy *et al.* (1989) as being the optimum for cellulolytic activity. The post feeding decreases in rumen pH were probably caused by the increased VFA production associated with consumption of the basal diet. Indeed pH followed a pattern inverse to that of total VFA concentration (Figures 6.1 and 6.3). Witt *et al.* (1999) reported a similar mean rumen pH of 6.5 in ewe lambs offered similar diets *ad libitum*.

6.4.3.2. Rumen ammonia.

The cyclical pattern of rumen ammonia concentration followed the pattern of feeding of the protein fraction of the diet, with lambs receiving the greatest quantity of rapidly degradable protein sources in a particular meal exhibiting the highest postfeeding peak in rumen ammonia concentration. Although the overall mean rumen ammonia concentration was 62.0mg/l, above the 50 mg/l recommended by Satter and Slyter (1974) as being the minimum required for maximum efficiency of microbial protein synthesis, this was due to the high peak values observed immediately post feeding. For most of the day the rumen ammonia concentrations were less than 50mg/l on all treatments which may have adversely affected microbial activity.

The rate of microbial utilisation of ammonia is affected by the availability of FME (AFRC 1995), and as the lambs in the current trial were offered a diet containing 55% barley on an *ad libitum* basis, the availability of FME to the microbes would be expected to be high enabling rapid utilisation and efficient capture of rumen ammonia resulting in low rumen ammonia concentrations. However due to the basal diet being withheld at the times of feeding the protein meals it is possible that in the first hour post feeding the release of ammonia in the rumen exceeded the capacity of the microbes to utilise it, resulting in the high peak values observed. Satter and Slyter (1974) observed that in situations where N is limiting to microbial growth fermentation becomes "uncoupled", in that fermentation and production of VFA continues at only slightly reduced rates, but that production of microbial N is severely restricted. In times of N under supply and carbohydrate excess some species of rumen bacteria have the ability to produce and store polysaccharide (Cheng *et al.*, 1973; Wells and Russell, 1994; Lou *et al.*, 1997). Sinclair *et al* (1995) reported that bacterial isolates from lambs fed asynchronous diets contained marginally more carbohydrate than those from sheep fed synchronous diets (144 vs. 140g/kg

microbial DM), but that carbohydrate source had a greater effect than synchrony on microbial carbohydrate reserves (Sinclair *et al.*, 1993). Some species of rumen bacteria also have mechanisms to decrease ATP production, for example via lactate production, or to "spill" the ATP already produced (Russel, 1998). The main mechanism of "energy spilling" appears to be an increase in the energy expended in maintenance of ion balance across the cell membrane (Russell and Cook, 1995). *Streptococcus bovis* has been shown to increase its non-growth energy disapation tenfold in conditions of excess glucose supply (Russell, 1991).

6.4.3.3. Rumen VFA's.

The hourly mean VFA concentrations in the current experiment varied from 33.1mM to 70.9mM, lower than the normal range of 70 to 130mM quoted by France and Siddons (1993) and also lower than the range of 45-75mM reported in Chapter 4 in restricted fed lambs. This reduction in VFA production may have been due to an uncoupling of fermentation due to carbohydrate being supplied in excess (Satter and Slyter, 1974). Witt *et al.* (1999a) fed diets similar to those used in the current experiment *ad libitum* and reported total VFA concentrations of between 38 and 45mM. The overall fermentation patterns were within the range quoted by Bergman (1990) with mean values of 58:33:9, 52:34:14, and 56:37:8 for acetate:propionate:butyrate for the synchronous, intermediate and asynchronous treatments respectively and were not significantly different between treatments. The major determinants of VFA absorption are rumen pH, outflow rate and fermentation pattern (Djikstra *et al.*, 1993). As intake and rumen pH were not significantly different between treatments it is probable that the quantity of each VFA absorbed was similar across treatments.

6.4.4. Plasma metabolites

6.4.4.1. Plasma ammonia.

Plasma ammonia concentration is a function of the quantity of ammonia absorbed from the rumen and the capacity of the liver to convert it to urea (Lobley *et al.*, 1995). The plasma ammonia concentrations in the current experiment broadly reflect the pattern of rumen ammonia concentration, with increases seen after consumption of the protein meals. However the highest concentration observed was 38μM, much lower than the peak value of 460μM observed in the experiment reported in Chapter 4, and the overall concentrations were much less variable. Peripheral plasma ammonia concentrations rise only when the livers capacity for detoxification has been exceeded (Linzell *et al.*, 1971; Symonds *et al.*, 1981; Orzechowski *et al.*, 1987). As significant differences between treatments were seen in rumen ammonia concentrations, but not in plasma ammonia concentrations then it would appear that ammonia absorption from the rumen did not exceed the capacity of the liver for detoxification. The sharp increase seen at 22.00h is difficult to explain as there was no equivalent increase in rumen ammonia concentration which might have caused an increase in plasma concentrations.

6.4.4.2. Plasma urea.

The range of plasma urea concentrations observed in the current experiment is similar to that reported by Witt *et al.* (1999a, b), and to those reported in Chapter 4. In agreement with Witt *et al.* (1999a, b) and Mandebvu and Galbraith (1999) the peaks in plasma ammonia concentration were seen within 3 hours of feeding the protein meals. The

tendency for lambs on the asynchronous diet to have higher plasma urea concentrations than those on the other two treatments would indicate that a greater quantity of ammonia was being detoxified in the liver of these lambs, with the associated increased inputs of amino acids and expenditure of energy (van der Walt, 1993; Lobley *et al.*, 1995). It is also possible that a greater quantity of urea was being recycled to the rumen in these animals as the quantity of endogenous urea transferred to the rumen is dependant on plasma concentrations (Nolan, 1993). Indeed, Holder *et al.* (1995) and Scollan *et al.* (1997) reported increased recycling of urea to the rumen on asynchronous diets compared to synchronous diets.

6.4.4.3. Plasma β-hydroxybutyrate.

The lambs in the current trial were receiving a diet high in barley on an *ad libitum* basis, so were highly unlikely to be in negative energy balance. In this case plasma β-hydroxybutyrate is more likely to have resulted from alimentary ketogenesis of butyrate in the rumen wall than from catabolism of free fatty acids mobilised form adipose tissue (Brockman, 1993). The lack of any differences between treatments reflects that there were no differences in rumen butyrate concentration. The overall pattern of β-hydroxybutyrate observed in the current experiment is similar to that reported by Witt *et al.* (1999a), although the significant differences between treatments reported by Witt *et al.* (1999a) were not seen in the current work. As previously discussed, the diets used by Witt *et al.* (1999a) differed in the main energy source within the diet, which may have given rise to the significant differences in plasma β-hydroxybutyrate concentrations. However it is also possible that the lack of a difference between the diets in predicted degree of synchrony of dietary N and OM release in the rumen, compared with the larger differences reported by

Witt *et al.* (1999a), may also have contributed to the lack of a significant difference in plasma β-hydroxybutyrate concentrations in the current work.

6.5. CONCLUSIONS.

In the current experiment, feeding lambs the energy component of the diet *ad libitum*, and altering the pattern of N supply to the rumen had no significant effects on growth or carcass characteristics. Rumen and plasma metabolites followed a cyclical trend between meals, but there were no indications of any alterations in either energy or nitrogen metabolism. However the difference in observed degree of dietary synchrony in lambs fed any of the three diets was very small.

CHAPTER 7.

THE EFFECTS OF DIETARY ENERGY SOURCE AND RUMEN SYNCHRONY ON GROWTH AND METABOLISM.

7.1. INTRODUCTION.

The current series of experiments were designed to investigate whether synchronising the hourly release of nitrogen and organic matter in the rumen would affect the growth and metabolism of lambs, without the confounding effects of differences in diet formulation encountered in some previously reported experiments (Herrera-Saldana *et al.*, 1990; Matras *et al.*, 1991; Aldrich *et al.*, 1993; Sinclair *et al.*, 1993; Witt *et al.*, 1999 a, b, Witt *et al.*, 2000). The first experiment involved characterisation of the feed ingredients to be used in the formulation of the diets in subsequent experiments, and enabled prediction of nitrogen and organic matter release in the rumen from these diets. The second experiment investigated the effects of nutrient synchrony on growing lambs fed at a restricted level, diets based on either rapidly or slowly degradable energy sources. The results from this experiment indicated that there were differences in energy metabolism due to both energy source and synchrony. Therefore the third experiment aimed to investigate whether these differences may have arisen from differences in diet digestibility, and also to assess whether there were any differences in microbial protein supply. The results indicated that energy source within the diet significantly influenced OM and NDF digestibility, and microbial protein production, but that the degree of dietary synchrony did not. The final experiment was designed to see whether the differences seen in the second experiment in

restricted fed lambs were also seen in lambs fed *ad libitum*. In this experiment no differences were seen between treatments.

7.2. EFFECT OF ENERGY SOURCE.

In the first growth trial lambs fed the barley based diet deposited almost 20% more fat in the whole body than lambs fed the sugar beet based diets. Lambs on the barley based diets did have a significantly higher GE intake than lambs on the sugar beet based diet (1690MJ *vs.* 1542MJ), but results from the metabolism trial would indicate that the digestibility of the OM within the barley diet was lower than that of the sugar beet based diet. The lack of any significant difference in total VFA concentrations, or in the total supply of lipogenic VFA (acetate plus butyrate) would indicate that the differences in fat deposition seen were not due to differences in the supply of lipogenic precursors from the diet. There was also no significant difference in propionate concentration, which would indicate that both energy sources resulted in a similar potential for production of glucose from this source. However the higher microbial protein supply seen in the barley fed lambs in the metabolism trial may have provided an increased post ruminal supply of amino acids, and in the absence of a higher N retention in these lambs it is possible that the carbon skeletons of these amino acids were utilised as energy sources. Also the sugar beet based diet was predicted to supply a greater quantity of DUP (39g/kgDM *vs.* 31g/kgDM for the sugar beet and barley based diets respectively), therefore the lower microbial protein supply on these diets may have been compensated for by a higher DUP supply.

Amino acids can act as precursors for the synthesis of glucose and glycolytic intermediates, and central to this process is the TCA cycle (MacRae and Lobley, 1984) (see Figure 1.3). The anabolism of acetate to fat has an obligatory requirement for both

NADPH and glycerol-3-phosphate (Annison and Armstrong, 1970). NADPH can be derived either from the pentose phosphate pathway, or from the isocitrate dehydrogenase shuttle (Smith *et al.*, 1983, MacRae and Lobley, 1984), whilst glycerol-3-phosphate is derived from the pentose phosphate pathway, the substrates for which, in ruminants, must come from either propionate or glucogenic amino acids (MacRae and Lobley, 1984). Therefore in situations where the supply of propionate is limited, amino acids are required to enable the synthesis of fat. Although in the first growth trial there were no differences between treatments in propionate supply, the greater postruminal amino acid supply provided by the higher microbial N flow seen in the barley fed lambs in the metabolism trial may have enabled more efficient anabolism of acetate to fat.

Higher nitrogen retention in the lambs on the sugar beet based diets was seen in the metabolism trial, but not in the first growth trial. The N balance results from the growth trial are likely to be more accurate than those from the metabolism trial as they are calculated from the actual N present in the body, rather than from the difference between N intake and output, which is subject to the inaccuracies inherent in collection of faeces and urine (Martin, 1966). The higher microbial N flow obtained in the barley fed lambs in the metabolism trial may have been expected to result in a higher nitrogen retention in these lambs. MacRae and Reeds (1980) concluded that in most situations the quantities of protein (microbial plus dietary) arriving at the small intestine had a greater influence on productivity than any aspect of protein quality. The lack of any effect on N retention would suggest that either there was a significant contribution of dietary protein to the post ruminal amino acid supply in lambs fed the sugar beet based diet, or that factors other than amino acid supply were influencing nitrogen balance. Plasma ammonia concentrations were significantly higher in the barley fed lambs in the first growth trial, but there were no differences in plasma urea concentrations between the two energy sources. The greater

inclusion of rapidly degradable N in the barley diet may have lead to the release of ammonia in the rumen exceeding the ability of the microbes for assimilation, as indicated by the high rumen and plasma ammonia concentrations. This may have lead to a greater requirement for detoxification of ammonia in the liver, and consequently the higher (although not significantly so) urinary N output seen in the metabolism trial.

Ammonia is known to have toxic effects within the body, and although the peak ammonia concentration seen in the current study (approximately 200μM) was well below that at which the signs of clinical ammonia toxicity are seen (between 500 and 800μM; Bartley *et al.*, 1981; Symonds *et al.*, 1981) it is possible that some detrimental effects on metabolism did occur. It is also known that hepatic detoxification of ammonia requires an input of amino acid (Lobley *et al.*, 1995), thus rendering them unavailable for productive purposes. This may also have contributed to the lack of difference in N balance seen in those lambs experiencing high plasma ammonia concentrations, despite an apparently greater supply of microbial protein.

It might also have been expected that the barley based diets would give rise to elevated plasma ammonia concentrations through the known detrimental effect of propionate on ammonia metabolism (Choung and Chamberlain, 1995), which is chiefly attributable to the inhibition of N-acetylglutamate synthesis (Stewart and Malser, 1980). However the lack of any differences in propionate concentration would suggest that this was not the cause of the increased plasma ammonia concentrations in the barley fed lambs in the first growth trial. There was no significant difference in plasma urea concentrations between energy sources, and as the quantity of N recycled to the rumen via saliva and direct diffusion is related to plasma urea concentration (Nolan, 1993), it can be postulated that N recycling would have been similar on both energy sources.

Both fat deposition and nitrogen retention are also under hormonal control. The secretion of both GH and IGF-1 are influenced by the level of nutrition of the animal (Breier, 1999). In the first growth trial, in which differences in fat deposition were observed, all lambs were fed at the same restricted level, However, the fact that GH and IGF-1 concentrations were not measures makes it difficult to speculate on any effect the different dietary regimes may have had on the secretion of these hormones. The lack of difference between energy sources in plasma insulin concentration indicates that the differences in fat deposition seen were not due to changes in insulin secretion.

7.3. EFFECT OF SYNCHRONY.

In the first growth trial synchrony appears to have had an effect on energy metabolism with lambs on the synchronous treatments depositing a greater quantity of fat around the kidney than lambs on the asynchronous treatments, who had a lower overall fat content. Whole body fat content was lowest in the lambs on the asynchronous treatment on both energy sources, but the highest fat content varied depending on energy source.

7.3.1. Barley based diets.

Within the barley based diets lambs on the synchronous treatment deposited the most fat in the body in the first growth trial, despite receiving identical quantities of each feed ingredient to lambs on the other two treatments. There were no differences in VFA production, and results from the metabolism trial revealed no differences in OM digestibility, so the apparent differences in energy metabolism were probably not due to differences in the supply of energy yielding nutrients from the diet. The results from the metabolism trial show a tendency for higher microbial N flows on the synchronous

treatment, and it is possible that the increased post ruminal amino acid supply on this treatment resulted in an increased supply of glucogenic and ketogenic amino acids, which were able to enter the TCA cycle and give rise to an increased supply of both glygerol-3-phosphate and NADPH allowing for more efficient anabolism of acetate to fat.

The lambs on the synchronous treatment also exhibited less variable plasma ammonia concentrations, with lower peak values than the lambs on the intermediate and asynchronous treatments. This indicates that there would have been a lower requirement for hepatic detoxification of ammonia in these sheep. As described previously, the conversion of ammonia to urea in the liver has been shown to be an energy requiring process (Lobley *et al.*, 1995), so it can be postulated that the hepatic energy expenditure may have been lower in the lambs on the synchronous diet, resulting in more energy being available for productive purposes.

In the second growth trial it is possible that the oversupply of energy from the basal diet was such as to mask any of these potential alterations in energy metabolism. The mean daily GE intake was 18.4MJ/d in the first growth trial, compared to 27.3MJ/d in the second. The total body fat content was the same in both experiments (5.07kg) but the lambs in the second growth trial took only 46 days to reach slaughter weight, whilst those in the first took 92 days. The higher intake of barley resulted in a higher molar proportion of propionate, allowing the potential for greater glucose synthesis from this source, and making any contribution from amino acid carbon skeletons less important. Also the plasma ammonia concentrations experienced by the lambs in the second growth trial were much lower and less variable than encountered in the first growth trial, so differences between treatments in hepatic energy expenditure for ureagenesis would have been small.

7.3.2. Sugar beet based diets.

The results obtained from the lambs on the sugar beet based diet are more difficult to explain. In the first growth trial the lambs on the intermediate treatment (SI) deposited more fat in the body than lambs on either the synchronous (SS) or asynchronous (SA) treatment (4.08kg *vs.* 4.76kg and 4.04kg on treatments BS BI, and BA respectively). This may be explained by the finding in the metabolism trial that the microbial N flow was marginally greater in these lambs, which may have subsequently provided a greater supply of energy yielding substrates, in the form of glucogenic and ketogenic amino acids, to the small intestine. However, the reasons for the higher microbial N flow on the intermediate treatment are not clear. Higher microbial N flow would imply a greater efficiency of N capture in the rumen, allowing greater quantities of microbial protein synthesis (Sinclair *et al.*, 1993, 1995; Herrera-Saldana *et al.*, 1990). However urinary N output was highest on the intermediate sugar beet diet, indicating the opposite. The first growth trial also showed no difference between sugar beet fed lambs in overall N balance, whilst the metabolism trial indicates that the lambs on treatment SA retained more N than lambs on treatments SI or SS. However none of these results reached statistical significance, so it appears that synchronisation of N and OM supply to the rumen has less effect when the diet contains a slowly degradable energy source than when the energy source within the diet is rapidly degraded.

7.3.3. Hormonal effects.

Within energy source, all lambs were not only fed at the same level, but also received exactly the same quantities of specific nutrients on a daily basis. This would make any differences in GH or IGF-1 secretion highly unlikely, as secretion of both hormones is under direct nutritional control (Brier, 1999). The lack of difference between treatments in

plasma insulin concentrations in the first growth trial would indicate that simply altering the pattern of N supply to the rumen has little effect on the secretion of insulin. However as neither GH or IGF-1 were measured, it is not possible to speculate as to whether the different dietary regimes had any effect on the secretion of the somatotrophic hormones.

7.4. CONCLUSIONS

Energy source within the diet had a greater effect on growth and metabolism of lambs than the degree of synchronisation of N and OM supply to the rumen. However synchronisation of N and OM supply to the rumen by altering the sequence of allocation of the feed ingredients did affect energy metabolism of lambs fed barley based diets at a restricted level. It is postulated that these effects may have been due to the combination of the effects of the increased entry of glucogenic and ketogenic amino acids into the TCA cycle, resulting in greater availability of lipogenic precursors, and the differences in the expenditure of energy by the liver in detoxification of ammonia. When offered a basal diet *ad libitum* intakes and growth rate were higher than predicted, and no effects of pattern of OM and protein supply on growth or carcass characteristics were seen. Whether the apparent increase in efficiency of energy utilisation seen in the restricted fed lambs can be exploited to produce commercial benefits is an area which requires further investigation.

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